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ERRATA

Volume V.

Page 596, line 10, for "nitric" read "citric".

Volume X.

Page 84, line 5, under "Methods", for "flame" read "frame".

Page 436, line 2, for "benzene" read "benzine".

Page 805, line 4 of footnote, for "which the" read "while the".

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THE EFFECT OF SORBED WATER ON THE PHYSICAL PROPERTIES OF ASBESTOS AND OTHER FIBRES, WITH SPECIAL REFERENCE TO RESILIENCE[1]

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Abstract

It has been shown that relative humidity exerts a definite effect on the physical properties of a mass of asbestos fibre. A special apparatus has been devised in which a mass of fibre may be compressed in such a way that the volume, pressure and relative humidity of the sample are known at any time. The effect of sorbed water on asbestos is to increase the apparent density and to decrease compressibility and resiliency. It has been assumed that the major factor which controls these characteristics is the fact that sorption of water affects the surface in such manner that the slippage of the fibres over one another is facilitated. Since no fibre unit is experimentally obtainable in the case of asbestos, the fibre bundle must be considered and the stiffness of such a bundle depends on the ease of slippage of its components and hence on relative humidity. This fact is of prime importance in the testing of asbestos where humidity will exert a direct effect on the screen test.

Cotton and wool fibres have been examined, and a definite effect of humidity demonstrated. Certain theoretical speculations in the case of cotton have been put forward.

Introduction

It has been known that sorbed moisture exerts a definite effect on the physical properties of fibrous materials, such as cotton; the effect of sorption on these organic fibres has generally been attributed to swelling and attendant phenomena. In the case of inorganic fibres such as asbestos, on the other hand, the physical effects of sorbed moisture are not as obvious, and until recently their existence had not been recognized. One of the writers (A.v.W) found that relative humidity exerted a definite effect on the physical properties of asbestos fibre. Screen test of the fibre showed definite variations and the apparent density seemed to be affected by humidity. In view of the practical importance of this observation, it seemed desirable to obtain quantitative data on the subject. The present research deals largely with asbestos fibre but has been extended to a limited degree to cotton and wool.

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An examination of the effects of sorbed moisture entails first, measurement of the amount of moisture sorbed under various conditions of relative humidity, and second, a measurement of the effect of this moisture on the physical properties of the fibres. The first part has already been carried out (2); the present paper describes measurements made on the second.

The physical properties of a fibre which control the behavior of a fibrous mass are: (*a*) structural, such as section modulus, elasticity, resiliency and shape; (*b*) surface, including coefficient of friction and contour, which influence the mutual effects of the fibres such as slippage and interlocking.

In order to determine the effect of humidity on these properties it is necessary to examine first the individual fibres and then their mutual effect as manifested in a mass of fibres. With asbestos, however, the problem is complicated by the fact that no fibre unit such as appears in the case of cotton and other organic fibres is obtainable. It is possible to subdivide asbestos fibres down to the limit of the microscope and even then there is no reason to believe that the process may not be continued. Hence the first part of the ideal research is beyond reach of present knowledge and technique, and it becomes necessary to deal with the fibres *en masse*.

One of the more obvious properties of a mass of fibres is the ability to be compressed and to return in some measure to the original volume when pressure is released. The amount of recovery after compression may be termed resiliency if the broader use of the word is accepted (*i.e.*, resiliency is the potential energy due to strain, without limitation as to exceeding the elastic limit). This property is determined by the structural and surface factors referred to above. Sorbed water may affect either or both of these, but in any case the change will be detectable as a change in the resiliency of the mass of fibres. Hence by measuring this resiliency at different humidities, quantitative data regarding the physical effects of sorbed water are obtainable. This procedure has been followed in experiments to be described.

Apparatus and Methods

An apparatus is required to compress a mass of fibres in such a way that the volume, pressure and relative humidity are known at any time. The method which has been followed consists of enclosing the fibre in a rubber bulb which is then subjected to a hydraulic pressure. A rubber bulb was previously used by Winson (5) when working with wool, but in his apparatus the sample was compressed by gas pressure. The change in gas pressure around the sample during compression offered a means of calculating the volume at any pressure from the gas law. This fact renders the apparatus unsuitable for the present use as changes in pressure in the gas surrounding the sample would result in changes in relative humidity. This difficulty has been removed in the apparatus to be described, in which the compression of the bulb is effected by the addition of a measured amount of liquid to a closed system. The volume and pressure may be directly and accurately measured. The pressure of the gas surrounding the fibres is unchanged during the compression, hence humidity remains constant.

Apparatus

The apparatus is illustrated in Fig. 1. A rubber bulb *A* containing the sample is placed on a brass tube *D* opening to the atmosphere through a conditioning chamber. The bulb is placed in a glass jar *B* which is fitted with brass end plates and tension bolts. A liquid-tight seal is obtained with a rubber gasket, fitted to the ground rim of the jar. This part of the apparatus is connected by suitable tubes to the volume and pressure metering part as shown in the diagram. The metering part consists of a mercury manometer *G*, designed to produce minimum volume changes and a burette for adding known amounts of liquid. The liquid was forced into the jar by displacement with mercury. Water has been found to be satisfactory though any other chemically inert liquid would function equally well.

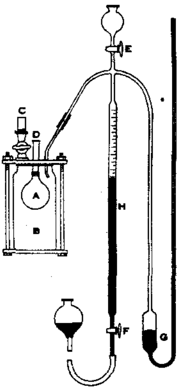

FIG. 1. *Diagram of compression apparatus.*

Method

a. Preparation and loading of samples. In the initial experiments the asbestos fibre was inserted in the bulb by hand, using blunt nosed forceps. A fine glass tube ran in through the neck of the bulb and extended to the opposite extremity. Air at known humidity was passed through this tube in order to condition the fibre. It was found that with asbestos, the gas flow through the fibre was localized, most of the flow apparently taking place in a channel around the inlet tube, hence other means of conditioning the fibre were adopted. A glass tube 3 cm. in diameter was drawn down in a long taper to a diameter of 1.5 cm. The fibre was placed in this tube and air at known humidity passed through for 24 hr. The rubber bulb was then fitted over the small end of this tube, and partly inflated by pumping conditioned air into the tube, the pressure required to inflate the bulb being 3-4 cm. of mercury. The asbestos fibre was then tapped into the bulb after which the pressure was slowly released while the bulb was tapped gently. In this manner a very uniform type of packing was obtainable, the apparent density of dry and conditioned fibre giving a close check between different loadings.

This loading technique could not be used with cotton and wool owing to the nature of the fibres. The dry samples were prepared in an oven at 105°C., and subsequently loaded into the bulb by hand using forceps, the bulb being placed in a desiccator into which a current of dry air was passed. The higher humidities were treated in a similar way in a stream of conditioned air. It would probably be feasible to condition the fibres of cotton and wool by passing conditioned air through the loaded bulb.

b. Manipulation of apparatus. After filling as described above, the rubber bulb is removed from the glass and the initial volume is obtained by water

displacement. The bulb is then fitted to the apparatus as illustrated, after which the apparatus is assembled and the end plates bolted.

The jar *B* is filled with water and care is taken to remove all air bubbles. The jar is then attached to the metering portion of the apparatus. Volume readings on the burette and corresponding pressure readings are taken. The compression is achieved by adding mercury to the apparatus through the lower stopcock *F*. The pressure may be released in a regular way by removing the previously added volumes. The rate at which equilibrium is established after displacement may be measured by taking pressure readings against time.

Results

Typical results appear in Tables I to VII. The volume of the sample is listed on the left with the corresponding pressure during each cycle of pressure and release appearing opposite. The initial volume is obtained by water displacement, and the subsequent volumes are based on it and include the volume change as read on the burette. The pressure readings were taken at a set time (15 sec.) after the corresponding volume change. Several runs were carried out in which 10 min. or more elapsed between each reading: though some pressure change took place during this time, the essential nature of the curves was unchanged, hence the shorter times were adopted for reasons of convenience. It was possible to check the results reasonably well between different runs. Small errors in the measurement of the original volume simply shift the whole curve without altering its shape. A standard maximum pressure of 48 cm. of mercury has been chosen. A number of experiments

TABLE I

Pressure-volume relation of asbestos fibre (30 gm.) dried in air for 24 hr.

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
81	0.0									
75	4.8									
69	9.8									
63	18.0									
61	21.6	0.1	3.2	0.1	1.2					
60	23.8	0.3	5.6	0.2	3.1	0.0	—	−0.1	—	−0.2
59	26.1	0.9	8.8	0.7	5.2	0.3	2.5	0.2	2.1	0.1
58	29.0	1.7	12.6	1.6	7.8	0.9	4.6	0.6	4.0	0.4
57	31.6	3.3	17.7	3.0	11.9	1.8	8.1	1.3	7.0	1.1
56	35.0	5.7	24.4	5.6	16.9	3.8	12.6	3.1	10.8	2.3
55	38.4	9.5	31.4	9.8	24.0	6.7	18.6	5.5	16.2	4.5
54	42.0	16.8	39.6	19.0	32.1	13.1	26.5	10.2	23.8	8.6
53	46.1	33.1	47.0	39.3	42.0	26.8	37.0	20.8	33.2	16.6
52.8	—	—	48.0	—	—	—	—	—	—	—
52.6	48.0	—	—	—	—	—	—	—	—	—
52.3	—	—	—	—	48.0	—	—	—	—	—
52	—	—	—	—	—	—	48.0	—	45.2	36.5
51.7	—	—	—	—	—	—	—	—	48.0	—

at lower pressures were carried out but, while they showed the same general effects, the curves were not quite as clear as at the higher pressure. The upper pressure limit of the present apparatus was 75 cm. of mercury.

The samples used in these experiments were of the following varieties:

Asbestos. This fibre was a well-fiberized standard mill product of the chrysotile variety obtained from one of the mines in the Thetford district in the province of Quebec, classified as 4M fibre and having a Quebec Standard Test of 0.0—4.0—8.0—4.0.

Cotton. Unbleached cotton wool, short fibre.

Wool. Scoured Colonial 56's.

TABLE II

Pressure-volume relation of asbestos fibre (30 gm.) in air at 95% relative humidity for 16 hr. Sorbed water 3.0% by weight

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
70	0.0									
64	9.4									
60	14.6									
56	21.8									
54	26.8	0.0								
53	29.4	0.2	2.9	0.0						
52	32.5	1.2	7.1	0.3	2.7	0.1		0.0		0.0
51	35.7	3.5	13.2	1.3	7.1	0.7	4.5	0.4	3.4	0.3
50	39.1	7.6	21.1	3.9	14.0	2.8	10.5	2.0	8.4	1.4
49	43.5	18.3	31.8	9.4	23.3	7.1	18.8	5.5	16.3	4.7
48	47.6		43.1	23.6	35.6	18.3	30.8	15.0	27.2	12.2
47.3			48.0		—		—	—	—	
47					48.1		45.6	39.6	43.0	33.0
46.8							48.0		—	
46.6									48.0	

TABLE III

Pressure-volume relation of asbestos fibre (30 gm.) in air at 60% relative humidity. Sorbed water 1.3% by weight

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
73	0.0							
68	6.1							
62	11.1							
56	20.1							
55	22.0	0.0						
54	24.2	0.3	3.4	−0.1				
53	26.7	1.2	5.0	0.1	2.5	0.0		−0.4
52	29.4	2.5	8.3	1.1	5.2	0.3	2.7	0.4
51	32.5	3.5	12.8		7.4	1.2	5.1	0.8
50	36.0	6.2	17.0	3.5	11.3	2.5	8.0	1.9
49	39.4		24.3	5.5	16.7	4.3	12.9	3.4
48	43.6	21.6	33.0	10.9	24.4	7.5	19.1	5.2
47.2	48.0							
47			42.0	22.6	34.5	15.0	28.1	11.3
46.2			48.0					
46					45.5	34.6	40.2	27.0
45.7					48.0			
45.3							48.0	

TABLE IV

Pressure-volume relation of unbleached cotton fibres (5.0 gm.) dried 24 hr. at 105°C.

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
51	0							
49	1.6	0						
47	3.5	0.3	0.5	0.0		−0.2		−0.3
45	5.6	0.6	1.9	0.5	1.1	0.3	0.9	0.2
43	8.0	1.2	3.5	1.0	2.7	0.9	2.4	0.7
41	10.5	2.5	5.4	1.9	4.4	1.5	4.0	1.6
39	13.2	3.7	7.5	3.1	6.3	2.7	6.0	2.8
37	16.5	5.2	10.3	4.5	8.9	4.9	8.3	4.0
35	20.2	7.0	13.6	6.1	12.0	5.8	11.4	5.4
33	24.7	9.8	18.2	8.7	16.2	8.0	14.0	7.5
31	30.1	13.8	24.0	12.6	21.9	11.7	20.8	11.2
29	37.2	21.6	31.6	18.7	29.5	17.0	28.2	17.0
27	45.4	36.0	42.0	31.8	40.2	28.8	38.4	27.2
26.4	48.0	—	—	—	—	—	—	—
26	—	—	48.0	—	46.5	39.8	45.0	38.0
25.7	—	—	—	—	48.0	—	—	—
25.5	—	—	—	—	—	—	48.0	—

TABLE V

Pressure-volume relation of unbleached cotton fibres (5.0 gm.) in air at 95–97% relative humidity for 18 hr.

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
52	0.3							
51	1.6							
50	2.7							
48	4.8							
46	6.6							
44	8.6	0.0		0.0				
42	11.1	0.4	1.6	0.4	1.2	0.1		−0.1
40	14.3	1.2	3.5	0.9	2.5	0.4	1.5	0.4
38	17.7	1.9	6.1	1.4	4.5	1.2	3.4	1.0
36	21.9	3.2	9.7	2.6	7.6	2.2	6.0	1.8
34	27.5	5.5	15.4	4.5	12.3	4.0	10.4	3.6
32	34.8	10.0	24.3	7.6	20.3	7.2	18.0	6.5
30	42.8	23.2	36.7	17.2	32.5	15.7	29.9	14.1
29	48.0		44.2	29.8	40.2	26.2	38.2	23.7
28.4	—		48.0	—	—	—	—	—
28.2	—		—	—	48.0	—	—	—
28.0	—		—	—	—	—	48.0	—

TABLE VI

PRESSURE-VOLUME RELATION OF WOOL (WASHED FIBRES, 35 gm.) DRIED AT 105°C.

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
56	0.6							
55	1.7	−0.2						
54	2.7	0.3	1.1	−0.1		−0.3		−0.4
51	5.2		2.8	0.6	1.4	0.5	1.4	0.4
49	5.8	1.5	—	—	—	—	—	—
47	7.5	2.2	4.5	1.7	4.0	1.5	3.2	—
45	9.0	2.8	5.8	—	—	—	4.5	2.0
43	10.8	3.6	7.5	3.2	6.4	2.8	—	—
41	12.9	4.6	9.3	—	—	—	7.2	3.5
39	15.0	5.6	11.3	5.3	9.7	4.6	—	—
37	17.5	7.7	13.5	6.8	—	—	11.2	5.8
35	20.4	9.5	16.2	8.3	14.5	7.9	—	—
33	23.7	11.8	19.7	10.7	—	—	17.0	9.8
31	28.2	15.3	24.2	13.7	22.1	12.9	—	—
29	33.7	20.3	29.7	18.5	27.6	17.3	24.6	16.6
27	40.8	28.9	37.4	25.5	35.0	24.3	—	—
26	44.9	35.5	41.4	31.4	—	—	38.1	29.0
25.1	48.0	—	—	—	—	—	—	—
25	—	—	46.5	40.0	44.8	37.6	—	36
24.6	—	—	48.0	—	—	—	—	—
24.3	—	—	—		48.0	—	48.0	—

TABLE VII

PRESSURE-VOLUME RELATION OF WOOL (5.0 gm.) IN CURRENT OF AIR AT 95% RELATIVE HUMIDITY FOR 24 HR.

Vol. cc.	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.	Comp.	Rel.
54	0.0							
53	1.0							
52	1.6							
51	2.1							
49	3.3	0.0						
47	4.9	0.1	1.1	0.0	—	−0.2	—	−0.2
45	6.2	0.4	2.3	0.2	1.3	0.2	0.8	0.2
43	7.2	1.0	3.5	0.7	2.5	0.4	2.0	0.4
41	9.0	1.8	4.9	1.6	3.7	1.3	3.0	0.7
39	11.7	2.7	6.7	1.8	5.1	1.8	4.4	1.5
37	13.4	3.4	8.6	3.0	7.1	2.6	6.0	2.6
35	16.3	4.8	11.2	4.2	9.5	4.0	8.3	3.7
33	20.0	6.4	14.9	6.0	13.0	5.2	11.6	5.1
31	24.8	8.8	19.7	8.8	17.5	7.9	16.1	7.2
29	31.2	13.7	26.5	12.7	24.0	12.0	22.5	11.0
27	40.1	22.2	35.4	21.5	34.0	—	31.8	18.8
26	45.0	32.2	—	—	—	27.8	—	25.2
25.3	48.0	—	—	—	—	—	—	—
25	—	—	48	—	47.6	—	46.0	36.7
24.6	—	—		—	—	—	48.0	—

Corrections

a. Pressure exerted by rubber bulb. The bulb was filled with air under known pressure and its volume measured by displacement.

The pressure exerted by the bulb varied from 1.1 cm. of mercury at 36 cc. volume to 2.2 cm. of mercury at 83 cc. volume (1.7 at 58 cc. and 2.1 at 69 cc.).

These pressures should be added to the pressure readings in all the preceding tables if absolute values are required. At present the work is solely of a comparative nature, hence this refinement has been omitted.

b. Volume change due to rubber connecting tube and capillary manometer. This was approximately 0.7 cc. for 48 cm. of mercury pressure rise. The value will be the same for conditioned or dry, hence a correction is unnecessary at present.

c. Pressure exerted by water column in left leg of manometer. At zero pressure in the jar *B* the right leg of the mercury manometer will register a slight pressure owing to the head of the water column over the mercury in the left leg. The manometer scale was therefore given a correction equivalent to this factor.

Discussion of Results

Asbestos

Pressure-volume relation. The experimental results have been summarized in Table VIII and in the accompanying graphs. In Fig. 2 the compression and release cycles for dry asbestos fibre have been plotted. It is

TABLE VIII

AVERAGE VALUES FOR VOLUME AT DIFFERENT CYCLES AND RELATIVE HUMIDITIES

Relative humidity, %	Initial vol., cc. 1st. cycle V_a	Initial vol., cc. last cycle V_b	Volume change in cc. between $p=o$ and $p=48$ cm. Hg.		$\frac{V_1}{V_a}$	$\frac{V_2}{V_b}$	Weight of samples, gm.
			1st. cycle V_1	Last cycle V_2			
Asbestos							
0.0	82.0	61.0	27.0	7.6	.330	.124	30.0
60.0	68.0	50.0	23.6	6.6	.348	.132	30.0
95.0	69.0	50.0	22.0	5.1	.319	.102	30.0
Cotton							
0.0	54.5	50.0	24.9	21.6	.457	.428	5.0
95.0	52.0	42.0	23.5	13.5	.452	.322	5.0
Wool							
0.0	56.0	54.0	30.9	29.7	.552	.550	3.5
95.0	54.0	47.0	28.7	22.4	.530	.478	5.0

apparent that the first compression results in a large amount of permanent deformation. The subsequent cycles continue the process but by the fifth cycle, the mass has nearly reached a position of equilibrium where the application and release of pressure will bring about repeatable volume changes. The ratio of volume change on the first and last cycles is seen in Table VIII (columns 6 and 7) (also Fig. 10).

It is of interest to note that the second compression curve falls well above the previous return, while a marked loop is formed owing to the fact that the return cycle shows very little volume change while pressure falls rapidly in the initial release. This indicates a "locked" condition after compression which prevents the volume from increasing until much lower pressures are reached. This state of affairs naturally follows when a deformation causes both bending of an elastic body and friction between this body and others.

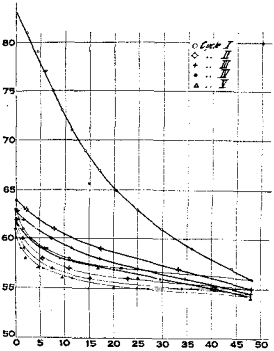

FIG. 2. *Pressure-volume relation of dry asbestos.*

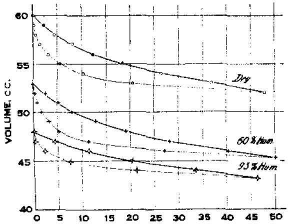

FIG. 3. *Effect of humidity on pressure-volume relation of asbestos fibre.*

A number of authors have obtained curves of this nature and have placed various interpretations on the areas.

Winson (5), investigating wool fibre, assumed that the loop areas in two cases, when volumes were plotted as percentage volume change, were a measure of the resiliency. This view is of course erroneous as it neglects the length of the loop and disregards the mechanical definition of resiliency.

Schiefer (3) in his work on compressibility of textile materials, regarded resiliency as the ratio of the work recovered from the fibre on the release stroke to the work done on the fibre on the compression stroke.

In the present work the stricter definition of resiliency will be adhered to. As mentioned previously, if the definition of resiliency be freed from the limitation of remaining within the elastic limit, the resiliency in this case is the work recovered as represented by the area under the release curves.

Effect of sorbed water. The aim of this research has been to demonstrate that sorbed water exerts a definite effect on the physical properties of asbestos

fibre. As a preliminary study three humidities have been chosen, *e.g.*, 0, 60, and 95%, corresponding to sorption values of 0.0 (dry at 104°C.), 1.3, and 3.0% respectively.

The effect of sorption is strikingly shown in the collected results in Table VIII, and in Fig. 3, where the compression and return curves for the final cycles of typical experiments have been plotted. The most striking effect is the large change in apparent density which results from changes in sorption. Numerical values for this difference in density are given in Table IX.

It is to be noted that the ratio of densities is similar at zero pressure for any cycle, while the actual value is directly related to humidity and thus to sorption. These differences of apparent density must be of great importance in any measurement which depends on density, such as a screen test, hence this factor will have a direct bearing on the commercial testing of asbestos.

Very much higher pressures would be required to compress the dry fibres to the same volume as the conditioned fibres. At the same time the percentage reduction caused by a given pressure is remarkably similar in all cases, and appears to be independent of sorption.

TABLE IX

VARIATION OF APPARENT DENSITY OF ASBESTOS FIBRE WITH HUMIDITY

Relative humidity, %	Initial cycle		5th cycle	
	Density (apparent)	Ratio	Density	Ratio
95	.455	100	.626	100
60	.435	96	.567	90
Dry	.371	80	.501	80

Resiliency. By previous definition work recovered during the release stroke is the measure of the resilience. This has been measured in arbitrary units using the planimeter and the results appear in Table X.

TABLE X

ASBESTOS FIBRE—WORK DONE DURING $p\ v$ CYCLE ($p\ v$ IN ARBITRARY UNITS)

Cycle No.	Dry fibre				Fibre conditioned at 95% relative humidity			
	Compress.	Release	Diff.	Ratio	Compress.	Release	Diff.	Ratio
1	2225	333	1992	.15	2170	285	1885	.13
2	759	300	459	.40	653	264	389	.40
5	601	245	356	.40	423	230	173	.53

Cotton wool

Pressure-volume relation. The pressure-volume curves for a typical sample of dry cotton wool are shown in Fig. 4. A curve very different from that for asbestos was obtained. The return curves fall much more closely on the compression curves and the difference between the first and subsequent cycles

is much less. The amount of locking of fibres is relatively less than with asbestos as the pressure does not fall nearly as rapidly on the release curves.

The dry samples are some 12% more resilient than those containing sorbed water, as will be seen by comparing the work done on release strokes. It is of interest to note that using Winson's definition (differences in Table X, a low value representing high resiliency) the dry fibre would be less resilient, but using Schiefer's (ratios in Table X) there would be no significant difference.

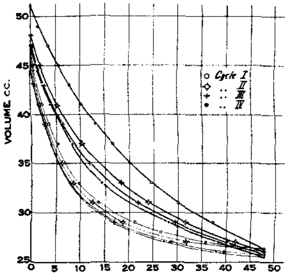

FIG. 4. *Pressure-volume relation of dry cotton wool.*

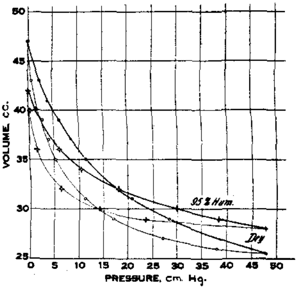

FIG. 5. *Effect of humidity on pressure-volume relation of cotton wool (final cycle).*

Effect of water sorption. In Fig. 5 are plotted the final cycles of cotton samples in the dry condition and in equilibrium with air at 95% humidity. In this condition, the cotton contains about 16% moisture, according to Urquhart (4). The curves may be best considered in conjunction with the values given in Table VIII.

There does not appear to be any difference in apparent density at the beginning of the initial stroke. This would be expected from the manner in which the cotton was packed in the rubber bulb. Considerable differences in behavior appear on compression, the conditioned sample being much less compressible, and though the loops are approximately the same size, the return loop for conditioned cotton shows an effect very similar to asbestos in which a small increase in volume entails a large drop in pressure, indicating a locked condition of the fibres (a sort of semi-permanent set).

The apparent density at the end of the compression stroke is reversed owing to the large compressibility of the dry fibre. Theoretical reasons will be advanced in a later part.

TABLE XI

COTTON FIBRE—WORK DONE DURING pv CYCLE (pv IN ARBITRARY UNITS)

Compression loop	4th cycle	
	Dry	95% relative humidity
Compression stroke	2116	1932
Release stroke	887	577

Values for resiliency appear in Table XI. The ratio of work done during release of the sample containing sorbed water to that of the dry fibre is lower than in the case of asbestos.

Wool

While outside the scope of this research, wool is of general interest as it exhibits a very twisted fibre and one likely to show a very high degree of resilience.

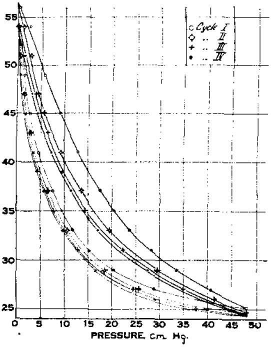

FIG. 6. *Pressure-volume relation of dry wool.*

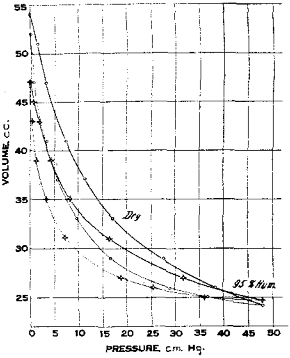

FIG. 7. *Effect of humidity on pressure-volume relation of wool (final cycle).*

Pressure-volume relation. The pressure-volume relation for dry wool fibre is shown in Fig. 6. It is at once apparent that much greater reversible volume changes are obtainable at the same pressure change than with cotton or asbestos. Practically no permanent set takes place (see also Fig. 10), the compression ratio on the first and final cycles being almost the same as shown in Table VIII.

Effect of sorbed water. The results of experiments on wool in the dry condition and containing sorbed water are shown in Fig. 7. The curves are not strictly comparable as the samples were not of the same weight. This discrepancy was unavoidable as the dry fibre occupied a much larger volume. However the effects of sorbed water are evident. The conditioned sample is less compressible and shows lower return and compression loops.

Comparison of Asbestos, Wool, and Cotton

In Figs. 8 and 9, typical pressure-volume curves for the fibres under discussion have been plotted for both dry condition and in equilibrium with air at 95% humidity. The volume changes have been plotted as percentages of the volume at the beginning of the last cycle. It is apparent that though asbestos

may be compressed only from 10 to 15% of its volume, wool may be compressed some 55% of its original volume by the application of the same pressure. Cotton falls between the two, showing 45% for dry fibre and 33% for fibre containing sorbed water. The effect of sorption has been in each case to reduce the compressibility of the fibre mass.

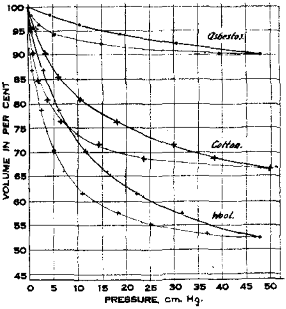

FIG. 8. *Pressure-volume relation of dry fibres (fourth cycle).*

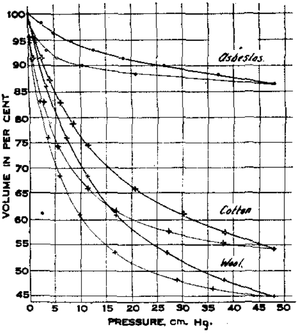

FIG. 9. *Pressure-volume relation of fibres at 95% relative humidity (fourth cycle).*

The pressure-volume curves have invariably been lowered by successive pressure and release cycles. This effect, which was greatest for asbestos and least for wool, is due to the fact that the fibres slip over one another to reach a position resulting in a smaller volume for the mass. The extent of this process is well shown in Fig. 10. In this graph, the pressure attained at a constant volume on succeeding cycles has been plotted against the appropriate cycle. As the fibres slip the pressure at any given volume falls with successive cycles. The differences between the fibres under discussion are at once apparent from this curve and the effect of sorbed water is very pronounced, particularly with asbestos and cotton. (Wool may not be directly compared owing to the weight discrepancy). Presumably one of the pronounced effects of sorbed water is to facilitate the slippage of the fibres.

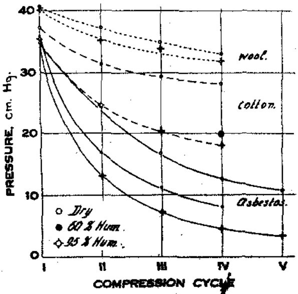

FIG. 10. *Effect of successive compression cycles on pressure (volume constant).*

It is, of course, apparent that the comparison between asbestos, cotton and wool may not be pressed very

closely because of the large differences in fibre length. When the length-volume relation is altered by shortening the fibre the resistance to bending is increased, which results in an increased tendency for slippage. This undoubtedly accounts for a part of the difference between asbestos and the other fibres which have been examined.

In general it seems that a definite measurable physical effect may be attributed to sorption by the organic and inorganic fibres examined. The effects may be summarized briefly as follows: (i) alteration of the shape of the compression and return loops; (ii) decrease in resilience; (iii) increase in apparent density; (iv) decrease in compressibility; (v) increase in the amount of permanent set or fibre slippage.

Theoretical Discussion

Asbestos

It has been clearly shown that the amount of sorbed water affects the pressure-volume curves in a regular manner. As indicated previously, this change in property may be due to alteration in the structural or surface characteristics of the individual fibre units. The first result would naturally follow sorption in which the molecules of water penetrated into the molecular structure of the sorbing medium. The second result would follow a purely surface sorption. It is suggested here that the results may be explained by assuming that the surface altered so that slippage of the fibres was facilitated.

As mentioned before, these factors could be separated if experiments could be carried out on the effect of moisture on the individual unit, but as this is impossible in the case of asbestos, the two effects must be treated together. There is, however, some evidence that only one of the factors is operative in the present case.

In the first place, it seems unlikely that absorption, or penetration of water into the molecular structure takes place in asbestos. This would presumably be a slow process while the authors have shown previously that sorption of water by asbestos, up to a humidity of 65% at least, is fairly rapid when disturbing factors, such as the presence of air, are excluded. This absorption effect might be expected with organic fibres where chemically bound water may be removed at fairly low temperatures; with an inorganic compound like asbestos, on the other hand, rather high temperatures are required to remove this water so that it seems unlikely that absorption takes place at the temperatures existing in these experiments.

It seems more probable that it is the surface factor that is being affected in the present case. The enormous subdivision of which an asbestos fibre is capable shows that a very large surface may be presented. The experimental results will therefore be discussed on the assumption that the effect of sorbed water has been confined to the surface and that the structural characteristics of the ultimate fibre unit are not appreciably changed. The differences which have been noted may be explained on the assumption that the major effect of sorption is to increase the ease with which the fibres slip over one another.

It has been shown that the application of a given pressure will compress the fibre containing sorbed water to a higher apparent density than the dry fibre. On the assumption which has been made, the ready slippage of the fibres containing sorbed water enables them to collapse over one another until a position of "repose" is reached, after which the mass may not be compressed further unless higher pressures are applied. Dry fibres will not slip as readily over one another; therefore, they are forced to bend.

When a mass of fibre is subjected to a volume deformation a change in apparent density follows, caused by the operation of two major factors, deflection of the fibres and slippage. The extent of deflection will depend on the bending moment to which each fibre is subjected. For any given force this moment depends on the unsupported fibre length between contacts with neighboring fibres. As the apparent density increases, the number of contacts increases, and the moment for a given force decreases. Hence the individual fibres are less deflected by a given force and the fibre mass becomes less compressible and resiliency falls. A simple case of this is illustrated in Fig. 11, which shows five fibres in a fundamental position.

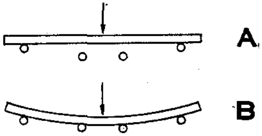

FIG. 11. *Effect of pressure on fibre deflection.*

In the initial stage *A* the fibre has insufficient strength to resist the stress; as it bends its resistance to deformation is increased by two factors, first, increased potential energy due to strain, and second, additional points of contact with adjacent fibres, which shorten the unsupported spans. This "stiffening" of the fibres means increased resistance to deformation, thus increasing the tendency for slippage. As this occurs more points of contact are formed and the compressibility of the mass falls, and apparent density increases. Therefore, it may be seen that if the average section modulus is unaltered, the pressure-volume relation of a mass of fibres is ultimately dependent on the ease with which they slip over one another.

If it is assumed that the effect of sorbed moisture is to facilitate this slippage, it follows that a mass of fibre in this condition will be more sensitive to compression, less resilient, and have a higher apparent density than a dry fibre.

In the above illustration, the simplest case of a horizontal beam has been chosen; actually of course, the reactions are enormously more complex, but the same general reasoning would apply.

This theoretical explanation appears to give a very satisfactory picture of compressional behavior of a mass of asbestos fibres. The shape of pressure-volume curve and the reason for apparent density differences which have been experimentally observed, are at once apparent. It has been noted that the resiliency of dry fibre is some 10% greater than for fibre containing sorbed water. This is predictable from theory, as the lower density and slip of the dry fibre allows a greater amount of volume change to take place by fibre bending. Hence, more work may be stored as potential energy.

Rate of Slip

Further evidence in favor of the explanation which has been offered is afforded by a measurement of the rate and amount of fibre slippage.

During the experiments it had been noticed that after a volume displacement some time elapsed before the pressure readings became constant. This is presumably due to slippage of the fibres resulting in a settling process. If the suggestions made above are valid, the process should be more rapid with fibre containing sorbed water. It was possible to measure the rate in the apparatus as employed. The pressure was rapidly increased at a constant rate to a fixed maximum, and pressure readings against time were then taken. The process was essentially that of measuring pressure change at a constant volume.

The first and second cycles of such a process appear in Fig. 12. It is at once apparent that both the rate and extent of slip are much greater for the fibre in equilibrium with air at 95% relative humidity. This appears to be very convincing evidence in favor of the theoretical explanation which has been put forward.

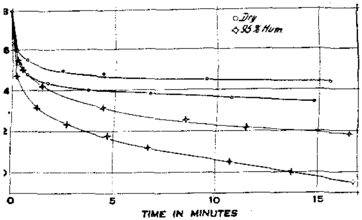

FIG. 12. *Effect of humidity on rate of fibre slippage.*

It might be suggested that the time required for the establishment of equilibrium was due to gas pressure in isolated pockets in the fibre mass. This is improbable as the time elapsed is sufficient for the entrained air to escape; furthermore, the order of magnitude of the observed effect would be reversed, as the rate of gas flow would be slowest through the fibre of highest apparent density.

Practical Applications in the Case of Asbestos

In the case of asbestos, the fibre bundle may be taken as the ultimate unit for practical purposes. The strength of this bundle of fibres will depend, among other factors, on the ease with which the individual fibres may slip over one another and as this is facilitated by the presence of sorbed water, a fibre bundle in this condition will be more flexible than such a bundle in the dry state.

This will have a direct bearing on the testing of asbestos. It is customary to screen the fibre through a number of standard meshes. It is obvious that at higher humidity, the long fibres will be more flexible and will pass through screens which would have held them, had they been dry. This fact has been noted in actual testing by one of the authors, but until now the reason has been in doubt. The difference in apparent density follows from the above and this will also exert a powerful effect on the screen test.

Only three humidities have been examined at present (0, 65 and 95%) so that the distribution of this effect over the range of sorption has yet to be elucidated. The sample at 60% relative humidity shows an initial apparent density similar to the samples at 95% relative humidity. Its behavior on compression is more like the dry fibre, while the loop falls half way between (see Fig. 3).

The properties which have been discussed must be greatly affected by fibre length but an examination of this factor involves a research in itself. It is possible that some relation between compressional behavior and fibre length might be obtained. Work of this nature is in projection.

Extension of the Discussion to Organic Fibres

That sorption affects the behavior of cotton and other organic fibres has long been known. The amounts of water which are taken up are much larger than those taken up by asbestos and the physical effects such as swelling, etc., are much more obvious. Hence, it would be expected that the physical properties of the individual fibres would be affected and perhaps also the surface characteristics as in the case of asbestos. Since a natural fibre unit of cotton is available it is possible to examine the surface and structural factors separately.

The first factor has been the subject of a number of researches. Clayton and Pierce (1) have shown that the elastic moduli of individual cotton hairs are influenced in a regular way by humidity. The resistance to both bending and torsion is reduced by sorbed water. It is therefore clear that the compressional behavior of a mass of cotton fibres will be affected by the alteration in section modulus caused by humidity.

It appears from the present results that the surface factor is also affected in such a manner as to increase the ease of slippage with increased amounts of sorbed water. While this effect is less marked than in the case of asbestos it is by no means negligible. This is seen in columns 6 and 7, Table VIII, where the average values for the ratio of volume change during compression to initial volume on the first and fifth cycles are 0.46 to 0.43 for the dry fibres and 0.45 to 0.32 for the conditioned fibres. Hence, considerably more packing has taken place in the latter case.

A combination of these two effects will readily explain the observed effects. In Fig. 4 it has been seen that while the apparent density of the dry fibre is lowest at the beginning of the compression it becomes greater than that of the conditioned fibre at the highest point of compression. This is probably connected with the swelling of the fibres on sorption of water. Clayton and Pierce (1) have shown that the volume of soda-boiled cotton is increased by some 12% by sorption of water from the dry condition to equilibrium with a humidity of 95%. Hence the swollen fibre may not be compressed as far as the dry fibre owing to this reduction in real density.

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THE ADSORPTION OF HYDROGEN SULPHIDE BY ACTIVATED ALUMINA[1]

BY C. H. BAYLEY[2]

Abstract

Measurements have been carried out on the adsorption of hydrogen sulphide on a commercial sample of "activated alumina" by the dynamic and static methods. The adsorption is influenced by prior heating of the alumina to various temperatures, being a maximum when the temperature of prior heating is 550° C.

The use of solid adsorbents for the removal of certain constituents of gaseous mixtures has received much attention in recent years. The use of silica gel in this connection is a familiar example. Aluminium hydroxide, when suitably treated, can be made to yield an oxide which possesses a high adsorptive capacity for many gases and vapors and a commercial grade of activated alumina suitable for such use has been on the market for some time.

The adsorption of a wide range of vapors by alumina has been investigated by Munro and Johnson (7). These authors emphasize the manner in which the adsorptive powers of the material are influenced by its water content. Boswell and Dilworth (1) have investigated the adsorption of ethylene on aluminium hydroxides containing known amounts of water, and have shown that there is, at a certain optimum water content, a maximum adsorption of ethylene. The use of "active" alumina as a desiccating agent has been investigated by Johnson (4) and others (2, 6).

The following are the results of an investigation upon the adsorption of hydrogen sulphide by one of the commercial grades of activated alumina. The extent of the adsorption of this gas from low partial pressure mixtures (0.5 to 2%) appeared to be of possible interest in connection with the purification of natural gas. Data were obtained regarding the extent of the adsorption of hydrogen sulphide from hydrogen sulphide-nitrogen mixtures and also in the absence of nitrogen by the static method. Owing to lack of time it was not possible to investigate the adsorption of hydrogen sulphide in admixture with the lower paraffin hydrocarbons. However, the results obtained are of interest, inasmuch as they reveal the existence of an optimum water content of the alumina which corresponds to maximum adsorptive activity.

Experimental

Materials

The hydrogen sulphide was obtained from a cylinder of the liquefied gas. Analysis by the usual iodine method showed it to have a purity of 99.6%. The sample of activated alumina was supplied by the Aluminium Corpor-

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Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.
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ation of America through their Montreal office and was designated by them as Grade A. The sample was ground to 20-40 mesh.

Apparatus

In the stream experiments, which were of a preliminary nature, the alumina was located at the centre of a 10 by 200 mm. Pyrex tube which could be heated electrically; dry nitrogen containing definite percentages of hydrogen sulphide as obtained by Jacobson's modification (3) of the method of Dunkley and Leitch, was passed, at a measured rate, over the alumina, the exit gases passing over a small piece of filter paper moistened with lead acetate solution, and thence through two gas washers containing a measured volume of standard iodine solution. In order to avoid loss of iodine, a third gas washer containing 10% potassium iodide solution was connected to the end of the train. The hydrogen sulphide taken up by the alumina was expelled into the iodine solution by prolonged passage of nitrogen or by raising the temperature of the alumina, and hence a check was obtained upon the amount taken as calculated from the volume of nitrogen-hydrogen sulphide mixture passed through. At the end of the experiment the contents of the three tubes were mixed and titrated with thiosulphate.

In the experiments employing the static method, the usual type of apparatus was used. This consisted essentially of a manifold carrying a calibrated bulb, burette, adsorption chamber, mercury manometer and outlet tube through which the system could be evacuated. Connection between the adsorption chamber and the arm of the manifold was made through a mercury seal.

In order to determine the volume of the apparatus, the calibrated bulb was filled with dry air and closed off from the rest of the system. A weight of alumina equal to that to be used in the subsequent experiments was introduced into the adsorption chamber, which, along with the manifold and manometer arm, was evacuated. The level of the mercury in the manometer was brought to the zero of the scale, and the pressure read. By opening the tap to the calibrated bulb and again reading the pressure, the volume of the adsorption system could be calculated. This method neglects any error arising from the adsorption of oxygen or nitrogen by the alumina, but this has been shown to be small. Pressures were read to the nearest millimetre of mercury and volumes to 0.1 cc. The temperature of the adsorption vessel was not regulated, being 22° ± 2° C. In the data, all gas volumes are corrected to 0° C. and 760 mm.

(a) Stream Experiments

Experiments were run with nitrogen-hydrogen sulphide mixtures of various compositions, the passage of the mixture being discontinued as soon as the lead acetate paper showed blackening. The hydrogen sulphide remaining in the reaction tube, as well as that adsorbed by the alumina, was then swept into the iodine with a current of nitrogen. In calculating the amount of hydrogen sulphide adsorbed, allowance was made for the amount of gas

remaining in the reaction tube. It was found that only a portion of the adsorbed gas was removed by nitrogen even after the latter gas was passed through for several hours. Upon raising the temperature of the alumina to 200° C., most of the hydrogen sulphide could be quantitatively recovered, but the amount recovered was always less than the amount used. This would indicate that a portion of the hydrogen sulphide had reacted chemically with the alumina. During the process, a small amount of moisture was formed and upon repeating the passage of the nitrogen-hydrogen sulphide mixture it was found that the extent of the adsorption, for a given partial pressure of hydrogen sulphide, had increased. The alumina was heated to 200° and 400° C. in a current of dry nitrogen and the adsorption measured after each heating. The results are shown in Table I.

TABLE I

ADSORPTION OF HYDROGEN SULPHIDE BY ACTIVATED ALUMINA, GRADE A. STREAM EXPERIMENTS

Weight of alumina, gm.	Rate of passage of gas mixture, cc./min.	Partial pressure H_2S, mm.	Temp. to which alumina was heated in N_2, °C.	H_2S adsorbed per gm. alumina, cc.
4.0000	10	224.0	Not heated	3.9
4.0000	10	52.5	200	5.4
4.0000	10	58.0	200	5.5
4.0000	10	67.1	400	6.1

(b) Static Experiments

Curve 1, Fig. 1, shows the results obtained with a sample of the alumina that had not received heat treatment. The desorption curve lies only slightly above that for the adsorption. The extent of the adsorption is relatively small, corresponding to 7.6 cc. per gram of material at a partial pressure of 714 mm.

Curve 2, Fig. 1, represents the adsorption on a similar sample which had been heated to 700° C. for two hours in a current of nitrogen, during which considerable quantities of water were evolved. The amount of adsorption is very much greater, being 20.5 cc. per gram of material at a partial pressure of 790 mm. In this case there was a well marked hysteresis of desorption

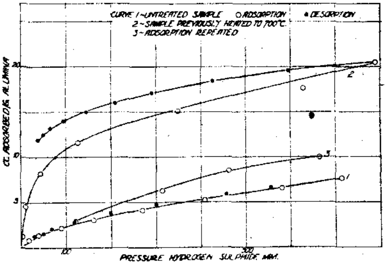

FIG. 1. *Adsorption of hydrogen sulphide by alumina before and after heating.*

and on lowering the pressure to 10 mm. there was still an unrecovered amount of hydrogen sulphide equal to 10 cc. per gram remaining on the alumina.

Curve 3, Fig. 1, shows the results obtained upon repeating the adsorption on the desorbed sample. The curve is similar in shape to Curve 2 but the extent of the adsorption is less than that shown in the latter by an amount approximately equal to the amount of gas that was not previously desorbed. Upon heating to 200° C. in a current of nitrogen, all of the gas held by the alumina was evolved and upon repeating the adsorption, the curve obtained coincided with Curve 2.

This observation, which was also made in the stream experiments, suggests that a portion of the hydrogen sulphide which is taken up reacts chemically with the alumina to form a compound which decomposes to liberate the gas on heating to 200° C. A yellow coloration developed on the alumina in these experiments. Its nature was not investigated.

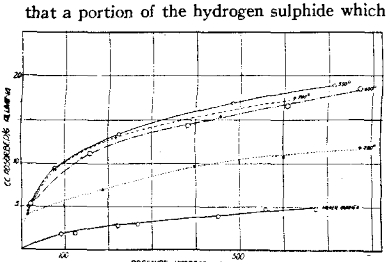

FIG. 2. *Adsorption of hydrogen sulphide by alumina heated to various temperatures.*

Fig. 2 shows the results obtained in a series of experiments in which 5-gm. samples of the alumina were heated respectively to 250°, 400°, 550°, 700°C. and strongly ignited with a Meker burner. The time of heating was three hours in every case.

For a given partial pressure, the extent of the adsorption steadily increases and reaches a maximum in the case of the sample heated to 550° C. In the strongly ignited sample, the extent of the adsorption has fallen to a value below that given by the unheated sample. This is undoubtedly due to the effect of sintering of the surface. It is probable that this effect would be noticed in a sample which had been subjected to prolonged heat treatment at 550° C. However, this appears to be the most suitable temperature to employ in the activation process provided the heating is not prolonged.

The water evolved in the various heat treatments was determined in each case. Table II shows the relation between the amount of water remaining on the alumina and the extent of the adsorption at three partial pressures. The total amount of water on the material was determined by measuring the loss in weight of a similar sample when heated to 1000° C. in an electric muffle, to constant weight. The optimum water content for maximum adsorption appears to be between 1.4 and 1.1% based on the original weight of the material prior to dehydration.

It will be observed that, for a given partial pressure of hydrogen sulphide, the adsorption values obtained in the stream experiments are lower than in the static experiments. This is due to the fact that in the former experiments

TABLE II

EFFECT OF WATER CONTENT ON ADSORPTION OF HYDROGEN SULPHIDE BY ACTIVATED ALUMINA, GRADE A

Material	Initial water/gm.	Temp. of heating in N, °C.	Water off, gm.	Residual water, gm.	Adsorption, cc. per gm.		
					50 mm.	100 mm.	400 mm.
Grade A	0.1035	400	0.0424	0.0611	7.4	12.0	14.6
Grade A	0.1035	550	0.0891	0.0144	8.4	13.0	15.7
Grade A	0.1035	700	0.0923	0.0112	8.4	13.0	15.3
Grade A	0.1035	280	0.0332	0.0703	5.5	6.7	10.2
Grade A	0.1035	Not heated	—	0.1035	1.5	3.5	5.0
Grade A	0.1035	1000	0.1035	—	1.2	1.8	3.8

the treatment was stopped as soon as hydrogen sulphide appeared in the exit gas and hence equilibrium between the gas and adsorbent was not reached.

The observation that silica gel and alumina which possess certain definite contents of water exhibit a maximum adsorptive power for various gases and vapors has been made by McGavack and Patrick (5), Munro and Johnson (7), and Boswell and Dilworth (1). These papers carry a full discussion of this phenomenon.

It should be noted that the value for the optimum water content of the activated alumina here investigated is considerably lower than that found by Munro and Johnson for the maximum adsorption of water vapor and by Boswell and Dilworth for that of ethylene. This is probably due to the fact that the activated alumina used here unquestionably possessed a smaller extent of active surface per gram than did that of the finely divided aluminium hydroxides used by the above investigators.

Acknowledgment

The writer wishes to express his thanks to Dr. G. S. Whitby, at whose suggestion this work was undertaken, for his permission to publish these results.

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INVESTIGATION OF VARIOUS PHYSICO-CHEMICAL FACTORS WHICH INFLUENCE SULPHITE COOKING[1]

By H. H. Saunderson[2] and O. Maass[3]

Abstract

The penetration of aqueous sulphite solutions into differently shaped blocks of spruce wood has been investigated, the effect of the shape and type of wood, and of the presence of imprisoned air being determined. Measurements of the rates of diffusion of the constituents of calcium bisulphite solutions into pre-soaked blocks of wood showed that "free" sulphur dioxide diffused more readily than calcium bisulphite. Adsorption of sulphur dioxide and calcium bisulphite solutions by spruce wood was measured at 30° and 50° C., and the influence of this factor on penetration was determined. The effect of penetration on delignification indicated the necessity of a satisfactory distribution of the lime salt at a low temperature to avoid localization of the lime during the pulping process.

Penetration

In a previous paper (9), the authors discussed the forced penetration of aqueous sulphite solutions into spruce wood, due to a difference in the hydrostatic pressure of the liquid on two sides of the piece of wood. The present paper reports an investigation on the penetration of aqueous sulphite solutions into wood blocks of various shapes where the solutions completely surround the wood. This approaches more closely to the conditions in the sulphite process of manufacturing wood pulp, and the results obtained may be applied to explain some of the features of that process.

Edwardes (1) found that with disks $\frac{5}{8}$, 1 and 2 in. in thickness, the penetration of liquid depended more on the pressure applied than upon any preliminary evacuation of the wood, a result contrary to that reported by Enge (2). Schwalbe and Berndt (10) compared the absorptions from equimolar solutions of calcium and magnesium bisulphites by blocks of wood averaging 40 by 7 by 2 mm. They found that the calcium and magnesium salts were removed to about the same extent, the amount of absorption being measured by weighing the soaked blocks. Schwalbe and Lange (11) found that heartwood and sapwood of spruce could be differentiated by their relative absorptions from magnesium bisulphite solutions. They found also that free sulphur dioxide solutions were absorbed more readily than those in which the sulphite was in the form of the magnesium salt. Richter (7) placed commercial spruce chips in bottles, added a definite volume of sulphite liquor, then observed the change in concentration with time.

In these various results, no attempt was made to separate diffusion from adsorption or chemical absorption. Such adsorption would be very important with regard to the penetration of sulphite liquor, but very little work has been done on the adsorption of either calcium bisulphite or sulphur dioxide

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by wood. Grace (3) studied the sorption of gaseous sulphur dioxide on small blocks of wood, and found that the amount of gas held increased continuously for 10 hr., the maximum being 13.6%. Of this amount, about 4% could not be removed by evacuation for 24 hr.

Natural penetration was studied in two ways, each of which would yield certain information. Sinkage tests were performed on various types and shapes of wood blocks under varying conditions. Richardson (6) found that practically a constant percentage absorption of solution was required to cause a block to sink, so the time a block took to sink was a measure of the rate of penetration of the liquor into it. The other method of approach was to study the concentration changes of sulphite liquor surrounding pre-soaked blocks of wood of various shapes. This gave an indication of the rate of diffusion of the solute particles into the wood. In order to estimate the effect of adsorption, some measurements were made of the amount of free and combined sulphur dioxide which could be adsorbed by wood. The effect on the pulp of the failure of the constituents of the liquor to penetrate satisfactorily was also tested in order to apply the penetration data to the problem of commercial sulphite "cooking."

Procedure

Sinkage Tests

Richardson's method for measuring the rate of penetration of liquids into various shapes of wood blocks was employed. Ten standard shapes of blocks were used, each being 2 gm. in weight. The measurements of the 10, measured longitudinally along the fibre direction were 2.5, 5, 7, 9.8, 15, 19, 32, 52, 90, and 128 mm. The transverse sections were square, of sufficient area to give a two-gram block. The apparatus used was a large vacuum desiccator, haviug an inlet tube through which liquid could be admitted quickly. A removable brass screen was arranged to fit about 2 in. from the bottom of the desiccator.

The samples of wood were placed on the bottom of the desiccator, then the screen fitted over them. The desiccator was evacuated for 15 min. using a water vacuum pump which reduced the pressure to about 10 mm. of mercury. Then sulphite liquor of the desired strength was allowed to run into the desiccator rapidly until it had filled all the region below the screen. Atmospheric pressure was restored, and the time it took the blocks to sink was noted.

Diffusion Tests

Blocks of wood of various shapes, previously soaked in water, were placed in sulphite solution, and the change in concentration of the sulphite liquor around the blocks gave a measure of its penetration into the wood. In addition to this simple diffusion, two other factors affected the sulphite concentration. The water in the soaked wood diffused outward into the solution, diluting it, and the wood surface acted as an adsorbent for both the free and combined sulphur dioxide.

The wood used was prepared in a manner similar to that used in the sinkage tests. Twenty blocks of approximately one gram each were made for each run, all blocks being of the same length, measured along the fibre. The weight of the 20 blocks was taken as a group. A sample of the same wood was used for a moisture determination, and from this the ovendry weight of the blocks was calculated. The blocks were carefully soaked in water by alternate evacuation and pressure for several days, by which time they were well filled. They were then rolled over blotting paper to remove surface moisture, allowed to stand in the air for 15 min., and weighed. Knowing the calculated ovendry weight and the weight of the wood when soaked, the amount of water introduced with the wood was determined.

The blocks were placed in a 125-cc. glass-stoppered reagent bottle filled with a measured amount of sulphite liquor of known strength. At intervals analyses were made of samples withdrawn from the bottle. The error introduced by the removal of this liquid was small, and no correction was made for it.

Adsorption Tests

The side arm of a 200-cc. Pyrex distillation flask was bent up parallel to the neck. A straight glass tube fitted through a rubber stopper down into the centre of the flask. This apparatus was connected to the original penetration apparatus (9, p. 418) by a heavy rubber tubing, clamped on. The feed tube from the penetration apparatus was connected to the side arm, the central glass tube leading to the outlet valve. The flask was fitted into the oil bath for temperature control.

The flask was filled with ovendry spruce flakes, then the central glass tube was worked down until the end of it was near the centre of the flask, and the rubber stopper was wired in place. The flask and wood were evacuated to a pressure of about 5 mm. of mercury, using a Hyvac pump connected to the outlet tube. Then the solution was allowed to flow into the evacuated flask. For the next 15 min. a gentle stirring motion was given to it by hand. At the end of 15 min., the outlet tube was flushed with about 15 cc. of liquid, and a test sample was withdrawn and analyzed.

Comparison of Runs 61 and 62 indicates that the dry heartwood sank much more quickly than the fresh-cut wood. This difference was due probably to a greater ease of removal of air in the evacuation of the dry wood.

The difference caused by evacuation of the sapwood was shown in Runs 63 and 64. In Run 64, in which the air was not removed, the time of sinking was several thousand times greater than that of the corresponding evacuated blocks. The relative order of sinking shows a slight change also. Where there was evacuation, the block that sank the most slowly was longer in proportion to its weight than the block from which the air had not been removed. Evidently the imprisoned air can diffuse out laterally, but the chief inflow of liquid into evacuated wood is along the tracheids.

TABLE I

RESULTS OF SINKAGE TESTS

SO_2 conc. in soln., %	Preliminary treatment	Length of block, mm.	Approx. cross section of block, sq. mm.	Time to sink	Length of block, mm.	Approx. cross section of block, sq. mm.	Time to sink
Run No. 61—Fresh heartwood. Temp., 25° C.							
Total, 4.99	Evacuation	2.5	1900	25 sec.	19.0	250	4 days
Free, 3.74	to 10 mm.	5.0	950	14.5 min.	32.0	150	6 days
Comb'd., 1.25	press. for 15 min.	7.0	650	69 min.	52.0	90	15 days
		9.8	480	5.5 hr.	90.0	52	16 days
		15.0	320	16.0 hr.	128.0	37	16 days
Run No. 62—Air-dry heartwood.* Temp., 25° C.							
Total, 4.99	Evacuation	2.5	2200	30 sec.	19.0	290	65.0 min.
Free, 3.74	to 10 mm.	5.0	1100	1.5 min.	32.0	170	44.5 min.
Comb'd., 1.25	press. for 15 min.	7.0	790	18.0 min.	52.0	105	6 hr.
		9.8	560	32.0 min.	90.0	61	11 hr.
		15.0	370	28.0 min.			
Run. No. 63—Fresh sapwood. Temp., 25° C.							
Total, 5.12	Evacuation	2.5	1500	20 sec.	18.8	200	75 sec.
Free, 3.84	to 10 mm.	5.0	750	20 sec.	32.0	115	270 sec.
Comb'd., 1.28	press. for 15 min.	7.1	535	20 sec.	51.0	75	345 sec.
		10.0	380	20 sec.	90.0	42	650 sec.
		15.0	250	35 sec.	128.0	30	405 sec.
Run No. 64—Fresh sapwood. Temp., 25° C.							
Total, 5.12	No	2.5	1500	9 hr.	18.8	200	16 days
Free, 3.84	preliminary	5.0	750	3 days	32.0	115	24 days
Comb'd., 1.28	evacuation	7.1	535	3.5 days	51.0	75	23 days
		10.0	380	4 days	90.0	42	18 days
		15.0	250	14 days	128.0	30	10 days
Run No. 65—Fresh heartwood. Temp., 50° C.							
		Length of block, mm.		Time to sink	Length of block, mm.*		Time to sink
Total, 5.00	Evacuation	2.5		30 sec.	19.0		9 days†
Free, 3.80	for 15 min.	5.0		15 hr.	32.0		10 days
Comb'd., 1.20		7.0		2 days	52.0		8 days†
		9.8		4 days	90.0		12 days
		15.0		7 days†	128.0		11 days

**This wood was not from the same sample as the wood used in Run No. 61, but had about the same number of rings per inch, and appeared to be of the same structure.*

†*At the end of five days the temperature was lowered to 25° C. The samples noted all dropped to the bottom, but when the temperature of 50° C. was restored, they rose again.*

TABLE II

RESULTS OF DIFFUSION TESTS

Wood	Vol. of sol. added, cc.	Conc. of sol. added, %	Equilibrium conc.* of sol., %	Time in min.	Conc. of the sol. around the wood: Free, SO_2, %	Conc. of the sol. around the wood: Combined SO_2, %
Run No. 71						
Type—fresh heartwood	75.5	Free SO_2, 3.33	Free SO_2, 2.36	5	2.72	1.22
Length, along fibres, 5 mm.		Comb'd. SO_2, 1.20	Comb'd. SO_2, 0.85	20	2.50	0.95
Oven-dry wt., 17.46 gm.				50	2.15	0.97
(calcd.)				110	2.02	0.90
Wt. after soaking, 48.36				235	1.99	0.89
gm.				470	1.97	0.85
Wt. of water in wood,				1435	1.94	0.81
30.90 gm.						
Run No. 72. Temp., 25° C.						
Type—fresh heartwood	81.6	Free SO_2, 3.35	Free SO_2, 2.46	5	3.07	1.13
Length, along fibres,		Comb'd. SO_2, 1.16	Comb'd. SO_2, 0.85	20	2.81	1.04
10 mm.				50	2.59	1.05
Oven-dry wt., 16.43 gm.				120	2.37	0.89
(calcd.)				240	2.13	0.93
Wt. after soaking,				480	2.10	0.83
46.02 gm.				1500	2.07	0.78
Wt. of water in wood,				6000	2.01	0.72
29.59 gm.						
Run No. 73. Temp., 25° C.						
Type—fresh heartwood	82.0	Free SO_2, 3.35	Free SO_2, 2.40	15	3.01	1.05
Length, along fibres,		Comb'd. SO_2, 1.16	Comb'd., 0.83	60	2.70	1.06
19 mm.				120	2.53	1.00
Oven-dry wt., 16.05 gm.				240	2.32	0.93
(calcd.)				480	2.14	0.93
Wt. after soaking,				1500	2.03	0.81
48.26 gm.				6000	1.95	0.75
Wt. of water in wood,						
32.21 gm.						
Run No. 74. Temp., 25° C.						
Type—fresh heartwood	87.0	Free SO_2, 3.33	Free SO_2, 2.48	30	2.86	1.22
Length, along fibres,		Comb'd. SO_2, 1.18	Comb'd. SO_2, 0.88	90	2.68	1.10
32 mm.				210	2.45	1.12
Oven-dry wt., 15.59 gm.				435	2.31	1.07
(calcd.)				1215	2.21	0.92
Wt. after soaking,				1905	2.14	0.87
45.24 gm.				2625	2.01	0.84
Wt. of water in soaked						
wood, 29.65 gm.						
Run No. 75. Temp., 25° C.						
Type—fresh heartwood	76.0	Free SO_2, 3.35	Free SO_2, 2.45	30	2.82	1.07
Length, along fibres,		Comb'd. SO_2, 1.16	Comb'd., 0.85	90	2.62	1.04
65 mm.				240	2.34	1.00
Oven-dry wt., 18.49 gm.				480	2.18	0.95
(calcd.)				1500	2.10	0.87
Wt. after soaking,				6000	2.02	0.78
46.37 gm.						
Wt. of water in soaked						
wood, 27.88 gm.						

**Assuming that there is no adsorption or chemical action which will remove sulphite from the solution.*

In heartwood, the least effective shape for absorption seems to be approximately 100 mm. long by 7 mm. by 7 mm. for a two-gram block when the wood is evacuated. To reach the centre, liquid from the ends would travel 50 mm. as compared to 3.5 mm. from the sides, which gives an indication of the relative velocities. No data were recorded for sinkage tests on heartwood where no evacuation was used.

The effect of a rise in temperature on the rate of sinking is rather remarkable. It is well known that diffusion is much greater at higher temperatures, so the increased time of sinking at 50° C. must be due to some other factor. The answer probably lies in the interesting test performed after Run 65 had been in progress for five days, when the solution was cooled to 25° C. The fact that three more blocks sank, and then rose again as the temperature was restored to 50° C. indicates that air pockets in the wood were keeping the solution out. The contraction of the gas as the temperature dropped sufficed to let the block absorb enough liquid to sink.

From these results, it is evident that the flow of liquids is much greater in a direction along the fibres than across them, but diffusion will take place across them.

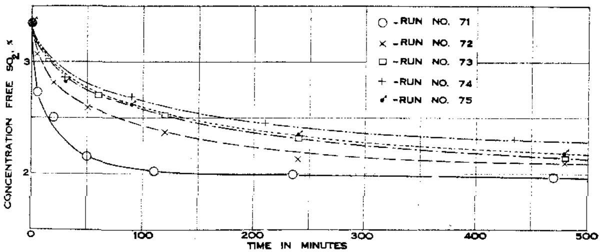

FIG. 1. *Rate of penetration of free sulphur dioxide for chips of different shapes.*

The results for the change in concentration of free sulphur dioxide in Runs 71 to 75 are shown graphically in Fig. 1.

In all the runs, the decrease in free sulphur dioxide was much more rapid than that of the combined. This may have been due to two causes; the free sulphite may diffuse more rapidly into the wood, or it may be adsorbed to a greater extent. The amount of adsorption is too small (see below) to account for all the change, so the free sulphur dioxide must diffuse more rapidly than the combined form. It is very probable that both factors contribute to the rapidity of the decrease.

The decrease of both free and combined sulphur dioxide in each run to a value below the calculated equilibrium was expected, owing to the known adsorption of sulphite on wood. The fact that free sulphur dioxide is adsorbed to a greater extent accounts for the greater drop below the equilibrium value.

A comparison of the five runs indicates the shape of block which removes the solute most slowly. For one-gram chips, this is the 32 mm. block, which apparently is the least effective shape to permit penetration by diffusion. It is noticeable that while this wood does not remove the sulphite rapidly, it continues to do so gradually over a long time period. The decrease in combined sulphur dioxide is proportionately not so rapid as that of the free in the early stages of a run, but toward the end of the run, its decrease is proportionately greater.

TABLE III

Adsorption tests

—	Run No. 46	Run No. 47	Run No. 48	Run No. 49
Temperature, °C.	30	30	50	50
Pressure, atm.	2	2	2	2
Weight of oven-dry wood, gm.	45.96	43.46	45.47	44.60
Volume of solution added, cc.	171	171	171	171
Concentration of solution added				
Free SO_2, %	1.98	1.94	1.97	1.84
Combined SO_2, %	1.38	1.39	1.38	1.41
Analysis of solution after 15 min.				
Free SO_2, %	1.87	1.82	1.90	1.79
Combined SO_2, %	1.35	1.37	1.36	1.39
Weight of free SO_2 adsorbed per gm. of wood, gm.	0.0041	0.0048	0.0026	0.0019
Weight of combined SO_2 adsorbed per gm. of wood, gm.	0.0011	0.0008	0.00075	0.00077

In general, the adsorption of free sulphur dioxide is about four times as great as that of combined sulphur dioxide. In both the temperature coefficient is negative, as would be expected for true adsorption, but the decrease is more marked with the free than with the combined sulphur dioxide.

The Relation of Penetration to Delignification

The practical effect of a lack of satisfactory penetration was investigated. In this work, the extent of delignification of the wood was accepted as an index of satisfactory cooking conditions. Four groups of experiments were arranged as follows:—*(a)* The effect of the forced penetration of ordinary sulphite solution through wood during the course of an ordinary "cook." *(b)* The effect of a rapid rise in temperature in the early stages of a "cook" on penetration and delignification. *(c)* The influence of penetration on the effect of cooking with sulphur dioxide solutions only. *(d)* The influence of penetration on the effect of cooking with lime solutions only.

The Effect of Forced Penetration

Three disks of air-dry heartwood, 2 cm. in thickness and 3 cm. in diameter, were made from one block of wood. The first of these was fitted into the apparatus for forced penetration (9) and was completely filled with sulphite liquor at room temperature. This disk was cooked for seven hours, the temperature rising to 110° C. in two hours, then gradually to 140° C. at the end of seven hours, a flow of about 3 cc. of liquor every 10 min. being maintained through the wood. At the end of seven hours, the wood was cooled rapidly, washed out with cold water, dried, and analyzed for lignin according to the method of Ross and Potter (8). The second block was filled with sulphite liquor by forced penetration at low temperature. It was then placed in the forced penetration apparatus and cooked loose, about 3 cc. of liquor being removed from around it every 10 min. The same range and duration of temperatures were used. The block was then analyzed. The third block was treated as the second, except that the preliminary filling was with water instead of sulphite liquor, the block then being cooked in sulphite liquor. Results are shown in Table IV.

TABLE IV

EFFECT OF FORCED PENETRATION ON DELIGNIFICATION

—	No. 51 Continuous penetration	No. 52 Preliminary sulphite penetration	No. 53 Preliminary water penetration
Lignin in product, %	13.13	17.41	22.95

NOTE.—*Concentration of solution: free SO_2, 1.89%; combined SO_2, 1.22%; pressure, 7 atm.*

Comparison of the three runs shows the effect of a supply of cooking liquor. Where there was a continuous fresh supply due to forced penetration, the delignification was more rapid, although in Run 52 the preliminary penetration of sulphite liquor assured the presence of the necessary reagents. The removal of reaction products in Run 51 probably contributed to the rate of delignification also. In Run 53, where the sulphite liquor had to diffuse into the wood, there was a much slower reaction. This established definitely the importance of satisfactory penetration.

The Effect of a Rapid Rise in Temperature

Two blocks of air-dry heartwood, 2 cm. in thickness and 3 cm. in diameter, were made from the same block of wood used in Runs 51 to 53. One of these was filled by preliminary penetration with a sulphite liquor which contained enough lime and sulphur dioxide to cook the wood, the amounts being calculated from Klason's figures (5). The other block was filled with water. The

TABLE V

EFFECT OF A RAPID RISE IN TEMPERATURE ON DELIGNIFICATION

—	No. 54 Preliminary sulphite penetration	No. 55 Preliminary water penetration
Lignin in product, %	11.73	31.26

NOTE.—*Concentration of solution: free SO_2, 2.87%; combined SO_2, 2.09%. Pressure, 7 atm.*

blocks were cooked loose in the usual type of penetration apparatus (9). The cell was filled with sulphite liquor, the temperatures raised to 140° C. in 18 min., and maintained for five hours. No fresh liquor was introduced during the run.

The apparently increased amount of lignin over that in the original wood is due to the decreased amount of cellulose and other constituents, which leaves a larger relative amount of lignin.

At the end of the cook in Run 54, the wood was lighter in color than normal wood, and was easily broken apart into fibres. The product of Run 55 was quite hard, and was brown in the centre. Along the ends of the block, the wood was of the same light color observed in the former run. This was evidently the only part delignified, while the centre was slightly burnt. Under the conditions of cooking used, the precipitation of a lime salt on the surface of the block probably let only a small amount of lime into the wood. This prevented the formation of calcium salts of the lignosulphonic acids, and the removal of the lignin in that form.

Run 54 showed that a rapid increase in temperature did not cause burning, provided that a sufficient supply of lime and sulphur dioxide was present. The slow temperature rise necessary in the usual mill cooks was thus proved to be due to lack of penetration rather than to the time required for any organic reaction to take place and thus "protect" the lignin.

The Effect of Cooking with Sulphur Dioxide Solutions

The effect of sulphur dioxide alone was investigated by making comparative runs following the same procedure as in runs Nos. 54 and 55, but using a solution of sulphur dioxide only. This solution was forced through one wood at room temperature, while the other block was being filled with water. Both blocks were then cooked loose in a cell for five hours at 140° C. after a rapid rise to that temperature. Both blocks were almost completely burnt, being a very dark brown or black. The wood had lost its mechanical strength and could be powdered very easily. There was no difference between the blocks.

TABLE VI

EFFECT OF COOKING WITH SULPHUR DIOXIDE SOLUTIONS ON DELIGNIFICATION

—	No. 56 Sulphite penetration	No. 57 Water penetration
Lignin in product, %	50.4	50.8

NOTE.—*Concentration of solution: free SO_2, 4.93%. Pressure, 10 atm.*

The Effect of Cooking with Lime Solutions

A corresponding pair of runs was arranged using lime only with no sulphur dioxide present. This presented one novel feature, a method of getting about 1% of lime into wood in the preliminary penetration. The method adopted was to penetrate the wood thoroughly with a 5% calcium chloride solution. Then the penetrating liquid was changed to 1% sodium hydroxide, and 45

c. was forced through the vood. This was just about the mount theoretically required o precipitate, as calcium ıydroxide, all the calcium resent in the wood as calcium hloride. Distilled water 100 cc.) was forced through o remove any excess of either alcium chloride or sodium ıydroxide. Only a small mount of lime would be washed out in this treatment.

TABLE VII

EFFECT ON DELIGNIFICATION OF COOKING WITH LIME SOLUTIONS

——	No. 58 Preliminary lime penetration	No. 59 Preliminary water penetration
Lignin in product, %	31.4	32.8

NOTE.—*Solution, saturated lime water. Pressure, 7 atm.*

The wood which had been penetrated with lime was slightly lighter in olor than the other, as though the wood which had been water-filled was urnt slightly. In neither case was there any evidence of cooking.

It is significant that in Runs 56 and 57, where no lime was present, there vas the same intensive burning of the wood that was only water-filled as of he wood filled with sulphur dioxide at the start. This proved that the ulphur dioxide, or its products that caused burning of the wood, was able o penetrate satisfactorily through the wood. When lime was present in ufficient amount, as in Run 54, the wood cooked normally. If only a small mount of lime was present, such as had probably diffused into the wood in Run 55, this small amount prevented the wood from burning to any extent, ut there was not sufficient to form soluble calcium salts of the lignosulphonic cids, and thus delignify the wood.

Discussion

The work on natural penetration indicated the ease of entry of liquid into vood in a longitudinal direction as compared with either tangential or radial low or diffusion. With regard to the shape of chips used in commercial ractice, it emphasizes the advantage of short chips, and shows the least ffective chip shape, variation from which in any direction will give more apid penetration. The best shape for penetration obviously will be one in vhich one dimension approaches zero.

The addition of sulphite liquor to the chips in the digester surrounds each hip with solution, imprisoning the air normally in the wood. A comparison f the sinking times of sapwood specimens of various shapes, evacuated and ot evacuated, showed that the air-filled wood took several thousand times s long to sink as a similar block evacuated. Though the pressure used in a ligester should reduce this difference very much, the presence of air in wood s a very important hindrance to penetration.

This factor is of importance in considering the relative merits of hot or old liquor for penetration. At a high temperature, any contained air will e in an expanded condition, occupying a larger part of the wood and allowing ess of the liquor to get in. One way in which this air may be replaced by

liquid is to get it into solution. At a high temperature, the solubility of gases in liquids decreases markedly, and the likelihood of the air dissolving lessens with rise of temperature. The imprisoned air at high temperature may exert its harmful effect in a third way. The free sulphur dioxide of the sulphite liquor will go into the gaseous phase readily, leaving the solution much weaker. If the vapor phase is large, relative to the volume of liquor in the wood, sufficient sulphur dioxide may escape, so that some calcium sulphite precipitates, clogging the wood and causing a maldistribution of the lime.

On the other hand, the use of a hot penetrating liquor has certain well-marked advantages. The entry of the cooking reagents is not only by the mass movement of liquid, but there is a diffusion of both sulphite and calcium ions into and probably through the wood. This diffusion will be hastened by a rise in temperature, the rate of increase being specific for each ion. Another advantage is that at a high temperature there is less adsorption of the reagents. The work on adsorption showed that at room temperature the concentration of the liquor as it advanced into the wood would be noticeably decreased, owing to the large adsorption of sulphur dioxide, and it would be altered in relative concentration owing to the difference in adsorption between free and combined sulphite. The work on forced penetration showed that a solution low in sulphur dioxide would not penetrate so readily, so there would not only be a decrease in solute concentration but a reduced amount of solution entering the wood.

A comparison of the conflicting lines of evidence shows that the objection to the use of hot penetration (other than the technical difficulties) is all based on the presence of air. This point should be given more thorough experimental investigation.

From the experiments on delignification, it seems evident that the chief problem in penetration is to ensure a sufficient supply of lime. The free sulphur dioxide penetrates without difficulty; according to the experiments on diffusion, it enters the wood much more rapidly than the combined sulphur dioxide. The chief obstacle to the penetration of an adequate amount of lime has been found to be precipitation. This precipitation of calcium sulphite at the surface of wood has been found to occur at temperatures much lower than was commonly supposed. The work of Gurd (4) on the equilibria existing in the SO_2-CaO-H_2O system gives more definite evidence of the precipitation temperature for varying concentrations. The precipitation point must not be exceeded in the early part of the process, or there will be localization of the lime, and consequent irregularity in the cook. This may be caused by local overheating or "boring." If the lime is distributed evenly through the wood, the temperature can be raised as rapidly as desired with no danger of burning. The presence of a large concentration of free sulphur dioxide prevents precipitation of the calcium salt, and also aids in its penetration. This is probably the reason underlying the recent use of solutions of high sulphur dioxide concentration.

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FURTHER INVESTIGATION OF THE PENETRATION OF LIQUIDS INTO WOOD[1]

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Abstract

The rate of penetration of water through heartwood, pressure and temperature remaining constant, shows an initial fairly rapid decrease followed by a gradual decrease to a constant rate. With unseasoned sapwood, the rate of penetration increases to a maximum, after which it slowly decreases. With seasoned sapwood the rate decreases with elapsed time. Pre-soaking for periods up to seven days does not affect the equilibrium rate of penetration. The initial rate is lower with pre-soaked than with air-dry wood. Pre-soaking does not hasten the attainment of an equilibrium rate. Rate of penetration is fairly constant for heartwood specimens of the same kind taken from the same transverse section of a tree. Unseasoned sapwood is more than 200 times as permeable as heartwood from the same tree. White spruce, black spruce and red pine heartwoods show about the same penetrability at low pressures. At higher pressures red pine becomes much more permeable than white spruce at the same pressure. The same is true of cedar, tamarack and balsam. Unseasoned sapwoods show increasing penetrability in the order hemlock, balsam, red pine, white spruce. Observed apparent penetration radially and tangentially through heartwood is less than 9% of that in the longitudinal direction. (Water actually penetrating was in no case greater than 1% of that penetrating longitudinally in the same time through a specimen of the same thickness.) In sapwood the rate of radial and tangential penetration is less than 2% of that in the longitudinal direction. Tangential penetration of white spruce sapwood is probably greater than radial penetration. Rate of longitudinal penetration increases with decrease in thickness of specimen. A very great increase in rate is noted after the thickness becomes less than one fibre length. This is suggested as a method of obtaining an approximation to the average fibre length. Rate is not inversely proportional to thickness, but decreases more rapidly with increasing thickness owing to loss of pressure head in passing pit membranes. Rate of penetration increases with increase in pressure differential. The rate is proportional to pressure (or slightly greater) in white spruce. The rate increases much more rapidly than proportionately with balsam, tamarack, cedar and red pine. This is due to bulging of thin pit membranes and consequent enlargement of perforations. Magnitudes of pressure and back pressure have no effect on rate of penetration, or time to reach an equilibrium rate, if pressure differential remains constant. Penetrability is not altered permanently by the application of pressure. Rate of penetration increases with temperature. Temperatures above 70° C. have a permanent effect on the penetrability. Intensive drying of wood increases its penetrability.

Sucrose solutions cause a decrease in rate of penetration greater than that expected from viscosity considerations. Molar sodium hydroxide increases the permeability of heartwood specimens of greater than one fibre length, and decreases that of sapwood and very thin sections of heartwood. Molar hydrochloric acid decreases the rate of penetration through heartwood, but has little effect on that through sapwood. Gases penetrate seasoned heartwood and sapwood easily. Pre-soaked heartwood strongly resists penetration by gases. Short lengths of unseasoned or pre-soaked sapwood are fairly easily penetrated by gases. All the evidence points to the absence of any valve action on the part of pit membrane tori.

A new theory has been advanced to account for the phenomena ordinarily ascribed to valve action, and also to explain those observations which could not be explained satisfactorily as due to torus valves.

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Economic Importance of Penetration

The permeability to liquids is a limiting factor in the choice of wood for many practical purposes. In some of the uses of wood, penetration of liquids into the body of the wood is of very great practical importance, and either optimum conditions for this penetration or means of eliminating or reducing it are of industrial value.

For cooperage purposes, where liquids are contained in wood for long periods, the wood should be practically impermeable to the contained liquid. White oak *(Quercus alba)* wood has been found to be almost impermeable to flow across the grain of the wood, and, since it also possesses other desirable properties, it is extensively used in this industry.

In many of its uses wood is exposed to the action of fungi and insects. Treatment of the wood for preservation against destruction by these causes is desirable. This involves impregnation of the wood to a greater or less extent by such substances as creosote or zinc meta-arsenite solution. When such treatment is to be given the choice of species of wood amenable to the treatment is important.

In lumbering operations, where logs are floated by water to the sawmill or pulp mill, great losses are sustained due to sinkage of logs *en route.* Logs of some species of wood are such poor floaters that they are not cut at all. In these cases, the use of methods by which penetration of water into logs could be sufficiently retarded to permit of a larger recovery of floated logs would greatly benefit the industry.

In the manufacture of wood pulp, the wood is disintegrated into fibres by mechanical or chemical means. The latter involves the loosening of the cementing action of the middle lamella by the use of suitable chemicals. Penetration of the cooking liquor into the wood is one stage of the process, and information as to the optimum conditions for this penetration would be of practical value to the industry.

Other cases might be cited showing the importance of penetration of liquids into wood, but the above are sufficient to show the economic value of information as to the permeability of various species, and of the factors affecting penetration of various liquids into wood.

Structure of Wood

In softwoods the predominant cell type is the tracheid (6). Tracheids are elongated cells, with tapering ends, extending parallel to the axial direction of the tree. The primary wall of a tracheid is formed by differentiation of living cells in the cambium layer of the tree. Secondary layers are laid down by apposition of new material, in the form of plates, centripetally to the primary walls. Tertiary walls may be laid down discontinuously on the secondary walls. The primary walls of adjacent cells become fused together forming what is commonly known as the middle lamella. The two most important constituents of cell walls of wood are cellulose and lignin. The

middle lamella is said (11) to contain a much larger proportion of lignin than do the secondary and tertiary walls, while the latter layers consist more largely of cellulose.

All the tracheids of a tree are not similar. In the season when growth is most rapid, the tracheids formed are relatively large, thin walled and loosely aggregated compared with those formed in the season of the year when growth is slower. These differing tracheids serve to mark out the annual rings of the tree. The thin celled part of the annual ring is known as springwood, and the thicker celled part, easily distinguishable by its denser structure and darker color, is summerwood. The transition throughout each annual ring is usually gradual.

In the original cells of the cambial layer communication between adjacent cells is provided by bundles of fibrils known as plasmodesma. These fibrils disappear when the tracheid is formed, but in the thickening of the cell walls, the areas through which fibril bundles passed do not thicken as does the rest of the wall. These thin areas, known as pits, with their perforations afford the means of communication between adjacent tracheids. In softwoods the pits are chiefly of the type known as "bordered" pits. In this type of pit the portion of wall separating the two lumina is the pit membrane. This membrane has a thickened central portion, the torus, and has numerous perforations in the marginal portion surrounding the torus. The secondary walls extend somewhat over the pit membrane but free from it, and have a perforation opposite the torus.

Adjoining tracheids overlap, at least the tapering portions where pits are usually most abundant. In softwoods, pits are largely restricted to the radial walls of the tracheids. They are so arranged that channels for longitudinal conduction of sap are provided through a series of lumina which form a more or less direct line.

The structure of wood outlined here is that given in most modern treatments of plant anatomy. Some writers differ in their view of the mode of formation of tracheids and in other details, but the authors consider this outline to give a simple and reasonable working idea of the subject.

It has been claimed that a pit membrane can act as a valve and close the pit by the torus being pressed against the pit mouth, and that this is a characteristic of water-conducting cells only.

There are two theories as to the change from sapwood to heartwood. One is that cells when first mature are most active in conduction of sap and there is a gradual slowing down of conduction until functional activity ceases, that is, it has become heartwood. The other theory is that the tracheids retain their conducting power until, for some reason, the pit membranes become closed and so prevent conduction of sap. Important changes taking place in this transformation are: the complete disappearance of protoplasts from the tracheid lumina; and withdrawal of cell sap; the water content of the cells is reduced; there are formed in, or brought into, the changing cells such new substances as oils, gums, resins, and coloring materials; and pit membranes

become fixed to a greater or less extent in the closing position. In this way the wood has become physiologically functionless and its only function is that of supporting the trees.

Other units of structure of softwoods are medullary rays and resin ducts. Medullary rays are rows of elongated cells extending in the radial direction of the wood. They have bordered pits by which they communicate with tracheids. Resin ducts are long, narrow intercellular spaces surrounded and limited by a layer of cells. They are filled with resin.

The structure of hardwoods is much more complex than that of softwoods. In the former, tracheids with bordered pits are absent, being replaced by wood fibres differing from tracheids by having thicker walls and narrow cavities, and simple pits. Vessels of indeterminate length extend as tubes longitudinally in the tree. They also occur as pores in cross sectional directions in the wood.

Researches on Penetration of Liquids into Wood

Early research on the subject of forced penetration of liquids into wood was undertaken by those interested in preservative treatment of wood. Tiemann (23) carried on some research on the subject and drew up an hypothesis to explain the mechanism of penetration of preservative liquids into wood. Microscopical examination of impregnated wood specimens led him to conclude that liquids penetrated chiefly by passing from one tracheid lumen to the next through minute slits produced in the walls of tracheids by seasoning. He thought, as did botanists at that time, that each tracheid of fresh wood was "completely closed by the continuous primary wall." The difficulty of forcing gases or preservative into fresh unseasoned wood seemed to be satisfactorily explained by this theory. Weiss (24) explained the greater penetration of preservative into summerwood than into springwood of the same annual ring by the fact that the thick walls of summerwood tracheids would crack more than the thin walls of springwood.

Teesdale (22) of the Forest Product Laboratories at Madison, Wisconsin, carried out tests on the amenability of 20 species of conifer to preservative treatment with creosote. He found that in species where there were radial resin canals, creosote was carried by these channels and from them penetrated longitudinally in the annual rings encountered. Here radial penetration was from one-fourth to three-fourths that in the longitudinal direction. Where there were no radial resin ducts, longitudinal penetration was from 20 to 120 times as great as that radially or tangentially. In all the common species, penetration into the late summerwood was more rapid than into other parts of an annual ring, but on standing a few weeks all parts of the annual ring became uniformly treated. In the pines, spruces and some other species, sapwood is much more penetrable than heartwood. This seemed to be the case in species which had a highly developed resin system, while species having no resin ducts showed little difference in permeability between sapwood and heartwood. In some species radial penetration was greater than tangential penetration, while in others the reverse was the case.

Bailey (1) undertook to check Tiemann's observations and theories. He used more refined microscopic methods in an examination of the structure of wood. His photomicrographs showed that the slits in tracheid walls, to which Tiemann attributed the means of penetration, were present in only about 10% of a large number of seasoned wood samples examined, and where present they did not extend through the primary wall. He found that though high pressures would not force air longitudinally through long lengths of fresh unseasoned wood, it was possible to force air through pieces of more than one fibre length. He was able to force a fine suspension of carbon through sections of wood, so concluded there must be some actual opening. Refined microscopy revealed the presence of perforations in the part of the pit membrane surrounding the torus. Photomicrographs showed carbon suspension passing from one tracheid to the next through bordered pits. These results disproved the theory of Tiemann and Weiss. He explained the difficulty of forcing air through fresh unseasoned or re-soaked, seasoned wood as being due to the surface tension of the liquid in the tracheid and on the application of further pressure the closing of the pits by tori being forced into a lateral position against the pit walls. The relatively smaller penetration in heartwood than in sapwood, he explains as being due to the filling of openings in the pit membranes with resins or tori being permanently cemented against the pit walls.

The greater penetration in late summerwood of heartwood is explained as being due to the small pits in those thick walled tracheids not being closed up as are those in springwood tracheids, which are easily forced into the lateral position, and so act as valves to prevent flow.

Bailey's contribution consists in the proof of the presence of perforations in pit membranes, and in the theory that mass penetration of liquids into wood takes place chiefly by passage from tracheid to tracheid through these perforations.

Observations from Research on Pulp

The part played by penetration in the initial stages of cooking in the manufacture of wood pulp is recognized by the industry. Miller (9) found that the production of a good sulphite pulp was dependent on complete impregnation of the wood chips by the bisulphite part of the cooking liquor, before the digester had reached the temperature of 110° C. He found that sulphurous acid penetrated more quickly than the bisulphite, and that lignin was rendered insoluble by the action of sulphurous acid alone, above 110° C. A proper co-ordination of chip length, penetration time, concentration of acid and pressure is of fundamental importance in the production of pulp.

Birchard (4, 5), noted that penetration in the longitudinal direction of the fibres is about nine times that in the other directions. He remarks that about the same relation, between penetration in different directions, is found in preservative impregnation of wood. These relative rates of penetration determine the most economical chip size. He mentions a case in a sulphite

mill where there were a large number of long slivers among the chips. These had not been completely penetrated when cooking temperature was reached, so were not cooked.

Research of Johnston and Maass on Penetration

Johnston and Maass (8) studied the time rate of entry of various liquids into prepared specimens of coniferous woods, and the rate of reaction between wood and cooking liquors, both under carefully controlled conditions. A suitable apparatus for this work was designed and constructed. Experiments were carried out on a wide range of subjects in order to test the adaptability of the method to the problem, and to make a general survey of the field of penetration of liquids into wood. In several cases inconclusive results were obtained. These were not sufficiently checked owing to lack of time.

Some of the results obtained for rate of flow of water through transverse sections were employed in a theoretical and quantitative consideration of the possible modes of flow through wood. Conclusions reached from this study were that all flow in heartwood did not take place through resin ducts, but that fibre penetration is important and is fitted to explain penetration as observed.

Some general conclusions drawn from experimental results were the following: (i) the rate of flow is characteristic of the species of wood; (ii) the flow in sapwood is about 100 times faster than in the corresponding heartwood; (iii) in heartwood, longitudinal flow is about 100 times faster than flow in the radial or tangential directions; (iv) in sapwood, flow in all directions is of about the same order of magnitude; (v) no tangential flow was observed in jack pine; (vi) the rate of flow diminishes with increasing thickness of section; (vii) water, at temperatures up to 140° C. has little permanent effect on the rate of flow; (viii) hydrochloric acid decreases the rate of flow very rapidly; (ix) in unseasoned wood and in sapwood rate of flow tends to increase in proportion to the pressure; in seasoned wood rate of flow increases more rapidly than applied pressure; (x) with increasing temperature, rate of flow increases more rapidly than fluidity; (xi) the rate of flow of water tends to reach a constant value which is not permanently changed by time, temperature or pressure; (xii) there is no evidence of tori acting as valves which are closed by the application of pressure.

Scarth's Researches

The study of wood structure in its relation to penetrability, from the viewpoint of the botanist, was continued by Scarth (13, 14). He prepared photomicrographs of sections of wood which had been treated with mercury under pressure. These photomicrographs showed that sapwood was completely impregnated with mercury, which was seen to have penetrated from lumen to lumen through pit membranes. In the case of heartwood the mercury was shown to have entered only to the ends of tracheids which had been cut. In all cases where penetration of mercury took place, the membranes were seen to occupy the median position in the bordered pits. Where

no penetration occurred, the membranes were in the lateral position against the pit opening, thus closing the pit by means of the torus.

Scarth and Spier (16) carried on further investigation by forcing water-soluble dyes into transverse sections. In spruce sapwood the tracheids in springwood were quickly stained, while in some cases summerwood tracheids were not stained in the same time. In heartwood, summerwood tracheids permitted penetration in a short time, but considerable time was required to stain springwood tracheids.

They found that boiling in water increased the permeability of spruce heartwood. Extraction with resin solvents, followed by boiling in water, increased the permeability only to the same extent as boiling water alone. From this they conclude that pit membranes have developed direct adhesion and fusion to the pit walls, rather than simply cementation by resins.

Scarth advances the theory that pit membranes are pulled over to the lateral position owing to the presence of colloidal material in the sap. After this has taken place the sapwood, presumably, becomes heartwood. He points out, however, that non-resinous conifers, *e.g.*, balsam, have a number of unclosed pit membranes in their heartwood, and so explains the greater permeability noted in these heartwoods. The observation is made that the application of pressure may close open pits by forcing the tori over against the pit walls, and hence, mere application of pressure may decrease permeability, unless the pressure be sufficient to cause rupturing of the membranes.

The penetration in spruce heartwood is explained as due to the pits in the summerwood remaining open, but having very small perforations. He remarks that summerwood in jack pine is almost totally devoid of effective pits.

*Stamm's Work on Structural Dimensions of Wood**

Stamm (17, 18, 19, 20, 21) of the U.S. Products Laboratories, Madison, Wisconsin, employed dynamic physical and electrical conductivity methods to measure structural dimensions of the capillary openings through wood.

He first used a method based on electro-endosmotic flow of water through wood sections. From data so obtained and applied in the formula for electro-endosmotic flow, he was able to calculate the effective capillary cross-sectional area of the wood as a percentage of the total cross-sectional area. This method also gave the fibre length of the wood, as when transverse sections were used, and the rate of flow was plotted against the thickness of wood, a sharp break occurred extending from the minimum to the maximum fibre length.

Measurements of the rate of hydrostatic flow through a wood in series with a standard glass capillary tube afforded data, which, when applied in

**Stamm's earliest work was published before that of Scarth or Johnston and Maass. The summary of Stamm's work follows the others in this introduction because reports of his work are still being published.*

Poiseuille's law in conjunction with the effective capillary cross section obtained from electro-endosmotic flow, permitted calculation of the average radius of the capillary openings.

Data on the maximum cross-sectional area of capillaries were obtained by the method of applying gaseous pressure to water soaked wood sections to a sufficient extent to permit of a flow of gas, by just exceeding the force exerted by the surface tension of water in the capillaries. Jurin's law was applied to the data so obtained to directly calculate the maximum radius of the pores.

A rough check on the results obtained by these methods was afforded by forcing a colloidal mercury solution, with particles of known size, through wood. To provide more accurate results, he devised an electrical conductivity method in which he measured the electrical resistance encountered by current in flowing through sections of wood impregnated with potassium chloride solution. Reasonably good agreement was noted between results by the electro-endosmotic flow method and those obtained from this conductivity method.

Apparatus and Technique Employed in Present Investigation

An apparatus previously used in a study of factors affecting penetration of liquids into wood was employed in a further study of the same subject. A number of changes was made in the apparatus in order to simplify procedure and to give greater accuracy in results. The most important change was the introduction of a new clamp for holding wood specimens during experiments. The procedure however has been adequately described elsewhere (8).

Scope of the Investigation

The investigation to be described in this paper is a continuation of the research of Johnston and Maass, with particular reference to mass penetration of liquids into and through wood specimens. The effect of time, temperature, pressure, wood structure and other factors on the rate of penetration of liquids into wood was studied.

The method of study was that adopted by Johnston and Maass. Specimens of wood, of the desired species, structural nature and size, were subjected to penetration by certain liquids in an apparatus where conditions of pressure and temperature could be controlled. A standard method of preparation of wood specimens was decided on after a few runs had been carried out, so that comparisons might be made between results obtained with different wood specimens. The possibility of duplicating results and the effect of pretreatment of wood specimens were tested. During the first part of the investigation experimental runs were carried out, using seasoned white spruce *(Picea canadensis)* specimens, to study the effect on the rate of penetration of the following factors: *(a)* pre-soaking of wood specimens for various lengths of time, *(b)* elapsed time of penetration, *(c)* various pressures and pressure differentials, *(d)* thickness of chip, *(e)* temperature, *(f)* three structural directions of wood, *(g)* sapwood and heartwood, *(h)* sucrose solutions, *(i)* hydrogen ion concentration and *(j)* intensive drying.

Following the study of penetration into seasoned white spruce, the investigation went on to unseasoned wood. Red pine *(Pinus resinosa)* balsam *(Abies balsamea)*, cedar *(Thuja occidentalis)*, tamarack *(Larix laricina)*, hemlock *(Tanga canadensis)* and white spruce have been studied. Factors examined using some, or all, of these species of unseasoned wood were: *(a)* variation with time of rate of penetration into heartwood and sapwood, *(b)* effect of thickness on the rate of penetration, *(c)* effect of pressure on the rate of penetration and *(d)* rates of penetration in the three structural directions of heartwood and sapwood. The effect of hydrogen ion concentration on the rate of penetration into unseasoned white spruce sapwood was also investigated. A number of runs was carried out to check up points relating to the fine structure of wood and to theories of the mechanism of penetration through sapwood and heartwood.

Experimental Work and Discussion of Results

Time-rate Curves

The first subject investigated was the variation with elapsed time of the rate of penetration of water longitudinally through white spruce heartwood. The penetration was carried out keeping driving and back pressures, and also temperature, constant throughout each run. In the first runs seasoned chips in the air-dried condition were used. These chips swell in the clamp, causing distortion and, in some cases, splitting of the chip. In order to avoid this difficulty, soaking of chips in water in an evacuated desiccator was tried as a pre-treatment. It was thought that this treatment might shorten the time required for the attainment of a constant rate of penetration, as well as eliminate distortion and breakage of chips in the clamp.

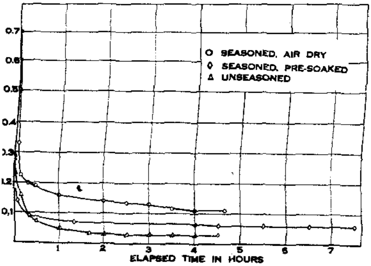

FIG. 1. *Time-rate curves for white spruce heartwood.*

Table I shows the variation with time of the rate of penetration through heartwood chips which had not been pre-soaked and also through some that had been given this treatment. Results from a run using unseasoned white spruce heartwood are also contained in the table. Fig. 1 shows graphically the variation with time of the rate of penetration for the same three cases. These curves are not to be compared with one another as to actual rates of penetration, since different pressures were used in the three cases. All three curves show that the rate of penetration rapidly decreases from the initial rate to a fairly constant rate which is attained in from two to four hours. The pre-soaked and unseasoned chips required about the same time to reach

TABLE I

VARIATION IN RATE OF PENETRATION OF WATER LONGITUDINALLY THROUGH WHITE SPRUCE HEARTWOOD—PRESSURE AND TEMPERATURE CONSTANT THROUGHOUT EACH RUN

Condition of chip*		Air-dry		Seasoned	Seasoned and pre-soaked 24 hr.		Un-. seasoned
Run No.		7	8	155	9	66	131
Pressure, lb./sq. in.							
Driving		25	25	40	26.5	70	60
Back		5	5	0	6	20	0
Differential		20	20	40	20.5	50	60
Elapsed time		Rate of penetration in cc. per min.					
Hr.	Min.						
	1			0.7			0.28
	2			0.5			
	3		0.18	0.37			0.23
	5			0.33	0.40	0.14	
	10	0.16	0.14	0.25	0.26		0.16
	20	0.12	0.12	0.20	0.24	0.09	0.09
	30	0.11	0.12	0.19	0.20		0.07
	40	0.08	0.10	0.18	0.18		
1	00	0.07	0.08	0.16	0.17		0.048
	20	0.05	0.07		0.16		
	40	0.048	0.06	0.15	0.14		0.034
2	00	0.048	0.053	0.14	0.12	0.067	0.031
	30	0.048	0.048	0.135	0.12		0.030
3	00	0.044	0.048	0.127	0.13		0.028
	30		0.047	0.116	0.12		0.027
4	00		0.049	0.111		0.064	0.027
	30		0.045	0.112		0.060	0.027
	32			0.12**			
	40		0.048	0.11			
5	00						
	30					0.060	
6	00						
	30					0.060	
7	00						
	30						
8	00					0.060	
	30						
9	00						
	30					0.059	

**All chips were ⅜ in. thick, except that used in Run 131 which was ¾ in.*
***Chip removed from clamp and replaced with other side up.*

this constant rate of penetration as did the air-dried ones. The chips classed as unseasoned really had become partially seasoned during preparation for use.

The rate of penetration, after a constant rate had been reached, expressed in cubic centimetres per minute was considered to be the equilibrium rate for the particular chip under the conditions employed. The equilibrium rate determined in this way was used in comparing the permeability of different chips under the same conditions or of the same chip under different conditions.

Wood substance absorbs water when in contact with it. This causes the wood to swell. This swelling causes a decrease in the size of tracheid lumina and pit membrane pores, thereby decreasing the rate of penetration.

This gradual decrease in rate of penetration cannot be satisfactorily accounted for by the hypothesis of tori being forced against pit mouths, and thereby closing the pores. Nor can it be explained as due to colloidal material from the inside of tracheid lumina being carried down and deposited in the pits. In Run 155 (Table I), the chip was removed from the clamp after equilibrium had been reached, and replaced so that water entered the side from which it had previously been flowing. The rate of penetration after resumption was observed to be unchanged due to this. In Run 160 (not tabulated), a spruce heartwood chip was penetrated by water until an equilibrium rate was obtained. The inlet copper tubing of the clamp was then exchanged with the outlet tube on the other side of the chip and penetration continued with pressures as before. The same equilibrium rate of flow was observed. On again exchanging inlet and outlet tubes the same equilibrium rate was again observed. A short lag was noticed in reaching the equilibrium rate after each change in direction of flow. This would not be the action of a torus valve. A back pressure just sufficient to prevent any flow through the chip was then applied and kept on for one hour. On releasing this back pressure, penetration resumed at the same rate as that before back pressure was applied. These results show that the decrease in rate is not due to tori acting as valves, nor to deposition of colloidal material in pit membrane pores.

The lumina of tracheids are tubes of capillary size. Pit membrane pores may also be considered as shorter capillary tubes of smaller cross section. Volume of flow of liquid through capillary tubes is proportional to the fourth power of the radius of the tube. Swelling of pit membranes and internal swelling of tracheid lumina with consequent reduction in size of passages through which water can pass is sufficient to explain satisfactorily the slowing down of flow during a run. The equilibrium rate is reached when a condition of physical equilibrium has been attained between wood substance and water.

Possibility of Duplicating Results

Before equilibrium rates of penetration, as obtained by the method outlined, could be used to compare the permeability of different species of wood, or of different specimens of the same species, it was necessary to test the range of variation between results obtained with similar chips under the same treatment. Some of the first runs carried out gave an indication that similar chips under the same conditions gave fairly reproducible results; *e.g.*, in Runs 7 and 8 (Table I) similar chips were used, and the rates of penetration observed were approximately the same. In Table II are given the equilibrium rates of penetration observed in a number of series of runs with similar chips used in each series.

The chips used in series 4a, 4b and 4c were taken from different parts of the same short (6 in.) log. Chips used in series 6a, 6b, and 6c were also taken from the same short log. Those in 6a were from near the heart pith, 6b about 2½ in. from the pith, and 6c, 3½ in. from the centre of the log.

The results of the various series show the degree to which observed rates of penetration are reproducible in similar chips. Also in all cases similar

TABLE II

EQUILIBRIUM RATE OF PENETRATION OF WATER THROUGH SIMILAR HEARTWOOD CHIPS AT ROOM TEMPERATURE

Series	Run no.	Thickness of chip, in.	Differential pressure, lb./sq. in.	Rate of penetration, cc./min.	Av. of series
White spruce					
1	27	3/8	20	0.029	
	28			0.035	0.032
2	44		50	0.130	
	45			0.129	
	46			0.154	
	47			0.082	0.124
3	48			0.039	
	49		50	0.040	0.040
4a	50		50	0.070	
	51			0.074	
	53			0.071	
	54			0.052	
	56			0.045	0.062
4b	55		50	0.065	
	57			0.048	
	60			0.079	
	61		50	0.059	0.063
4c	62		50	0.080	
	63			0.067	
	65			0.083	
	66			0.060	
	67			0.068	0.072
5	128	3/4	60	0.022	
	130			0.023	
	131			0.027	0.024
Balsam					
6a	119	3/4	20	0.39	
	120			0.29	
	121			0.30	0.33
6b	114		20	0.26	
	115			0.40	
	116			0.26	0.31
6c	122		20	0.29	0.29

chips show a reasonably close agreement in rate of penetration. Further examples of the degree of reproducibility in results are shown in Table VI. The series from different parts of the same log show that in heartwood the permeability is of the same order of magnitude regardless of the distance from the centre of the log. This point, however, has not been sufficiently investigated to warrant definite conclusions.

The results obtained with white spruce chips show a considerable range of variation in penetrability among specimens from different trees. The difference in penetrability between white spruce and balsam is much more marked.

Effect on Penetrability of Pre-soaking of Chips

Though pre-soaking of seasoned chips had not materially decreased the time required for attainment of an equilibrium rate of penetration, yet it was found necessary to carry out this pre-treatment to avoid breaking and distortion of chips in the clamp. The effect of pre-soaking for various times, ranging from one to seven days was tested. Three series, of four similar chips each, were employed. These chips were soaked in water in a desiccator which was evacuated occasionally.

TABLE III

EFFECT OF PRELIMINARY SOAKING OF CHIPS ON THE RATE OF PENETRATION

Run no.	Period of preliminary treatment in water, days	Rate of penetration in cc./min.
13	1	0.062
11	3	0.092
12	5	0.075
14	7	0.041
15	1	0.030
16	3	0.030
17	5	0.018
18	7	0.035
19	1	0.018
20	3	0.018
21	5	0.020
22	7	0.022

Thickness of chips, $\frac{3}{8}$ in.; driving pressure, 70 lb. per sq. in.; back pressure, 20 lb. per sq. in.; differential pressure, 50 lb. per sq. in.; temp., 23° C.

Table III gives the results obtained for the three series of chips. The chips used in the first two series (Runs 11 to 18) were sawed off a cylinder of the required size, and were not smooth in the manner already described. The results obtained in these two series show large variations compared with the close agreement among those obtained in the third series (Runs 19 to 22) and also in the different series of runs listed in Table II.

In all these runs a constant rate was reached in about 3 hr., hence no time would be saved by prolonging the period of soaking beyond 24 hr., as this period of soaking swelled the chips and softened them sufficiently to prevent breaking in the clamp. Pre-soaking for 24 hr. was therefore adopted as preliminary treatment for seasoned chips.

Influence of Pressure on Rate of Penetration

The effects of pressure differential, and of magnitude of pressure on the rate of penetration were studied. Three runs were carried out using different driving and back pressures while maintaining a constant pressure differential. These runs were made at room temperature with water as the penetrating liquid. The results obtained are tabulated in Table IV. These runs show that the rate of penetration is dependent on the pressure differential and not on the magnitude of the pressures used.

A series of three runs was carried out to find if the magnitude of the pressure affected the time required to reach a constant rate of penetration. Unseasoned white spruce heartwood chips $\frac{3}{4}$ in. thick were used. The pressure differential was 20 lb. per sq. in. Results obtained for rates of penetration and time to reach equilibrium are shown in Table V.

These results show, as have nearly all runs carried out at room temperture, that a penetration time of from 3 to 4 hr. is required to reach a constant

TABLE IV

EFFECT ON RATE OF PENETRATION OF MAGNITUDE OF PRESSURE WITH PRESSURE DIFFERENTIAL CONSTANT

Run no.	Driving pressure, lb./sq. in.	Back pressure, lb./sq. in.	Differential pressure, lb./sq. in.	Rate of penetration, cc./min.
9	26.2	6	20.2	0.103
	46	25.2	20.8	0.098
	26.2	6	20.2	0.100
10	26.2	6	20.2	0.064
	45.5	25	20.5	0.064
70	40	0	40	0.112
	47.5	7.5	40	0.115
	55.5	15.5	40	0.113
	64.5	24.5	40	0.107
	70	30	40	0.115

TABLE V

EFFECT OF MAGNITUDE OF PRESSURE ON TIME TO REACH AN EQUILIBRIUM RATE OF PENETRATION

Run no.	Pressure in lb./sq. in.			Time to reach equilibrium, hr.	Rate of penetration, cc./min.
	Driving	Back	Differential		
128	20	0	20	3½	0.0068
132	40	20	20	4	0.0070
133	60	40	20	3	0.0065

rate of longitudinal penetration regardless of pre-treatment or pressures used. The close agreement in these equilibrium rates of penetration furnishes further evidence that magnitude of pressure does not affect the rate of penetration provided the differential pressure is maintained constant.

The effect, on longitudinal rate of penetration, of varying the pressure differential was shown in numerous runs on heartwood chips of the following species: seasoned white spruce, unseasoned white spruce, unseasoned black spruce, unseasoned cedar, unseasoned red pine, unseasoned tamarack, unseasoned balsam and unseasoned white pine.

The rate of flow longitudinally through wood would be expected to vary directly with the pressure head applied, if the openings remained con-

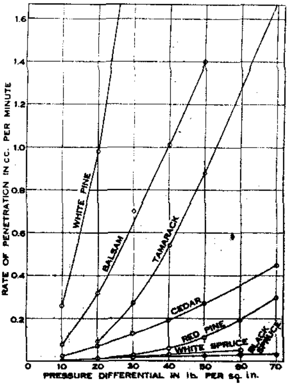

FIG. 2. *Variation with pressure of longitudinal penetration through heartwood.*

TABLE VI

EFFECT OF PRESSURE DIFFERENTIAL ON RATE OF LONGITUDINAL PENETRATION OF WATER AT ROOM TEMPERATURE

Run no.	Thickness of chip, in.	Rate of penetration in cc/min. at the following pressures in lb. per sq. in.						
		10	20	30	40	50	60	70
White spruce (seasoned)								
23	3/4		0.013		0.026		0.035	
30	3/4		0.018		0.044		0.062	
32	3/4		0.009		0.026		0.070	
27	3/8		0.029		0.069		0.119	
28	3/8		0.035		0.095		0.190	
31	3/8		0.031		0.088		0.194	
White spruce (unseasoned)								
128	3/4		0.007		0.015		0.023	
130	3/4				0.014		0.023	
Black spruce (unseasoned)								
172	3/4	0.003	0.010	0.014	0.022	0.025	0.030	0.032
173	3/4	0.003	0.011		0.025		0.040	
Cedar (unseasoned)								
170	3/4	0.044	0.116	0.20	0.31	0.41		0.64
171	3/4	0.022	0.06	0.11	0.17	0.24		0.42
169	3/4	0.018	0.043	0.075	0.12	0.16		0.29
Red pine (unseasoned)								
109	13/16		0.011	0.03	0.06	0.11	0.19	0.30
105	7/16		0.08	0.23	0.46	0.56		
Tamarack (unseasoned)								
166	3/4		0.107	0.33	0.67	1.07		1.86
168	3/4		0.075	0.20	0.41	0.68		1.52
Balsam (unseasoned)								
114	3/4	0.05	0.26	0.58	0.79	1.00		
115	3/4	0.09	0.40	0.90	1.40	1.8		
116	3/4	0.05	0.26	0.56	0.75	0.95		
119	1/4	0.13	0.39	0.86	1.25	1.9		
121	3/4	0.07	0.30	0.67	1.06	1.3		
122	3/4	0.08	0.29	0.60	0.82			
176	1½	0.028	0.11	0.20	0.31	0.42	0.51	0.60
White pine (unseasoned)								
174	3/4	0.26	0.98	2.25	3.80			

stant in size. The results in Table VI and the curves in Fig. 2 show that with white spruce and black spruce the rate is approximately proportional to pressure. However, with the other species of wood the rate increases much more rapidly than in direct proportion to pressure head.* This must indicate some physical effect of pressure acting so as to change the size of the openings through which water is passing. This effect of pressure is explainable as due to the action on pit membranes through the pores of which liquid is flowing. These thin membranes are probably bulged out somewhat from their median position, thus enlarging the pores and so permitting more rapid flow.

Another possible explanation would be that the increased pressure might enlarge perforations by forcing water out of the membrane. This explanation, however, is ruled out by the results obtained with constant pressure differential with varied magnitude of driving and back pressures. Magnitude of differential pressure alone was found to effect the equilibrium rate.

Plant anatomists point out that in balsam heartwood a relatively larger number of pit membranes are in the median position than is the case in the other species tested. They also say that in spruce heartwood, practically the only pit membranes remaining in the median position are those in the latest summerwood of the annual rings. This is also the case in the heartwood of most other conifers. But in balsam heartwood about 10% of all the pits have their membrane still in the median position. It follows from these facts that many of the open pits in balsam heartwood are in thin walled tracheids. The membranes of such pits would be stretched to a greater extent than would the thick membranes of the open pits in spruce, and hence perforations in balsam membranes would be enlarged to a greater extent by pressure. A similar condition probably occurs in tamarack heartwood.

It may be that the membranes of open pits in red pine and cedar are thinner than those in spruce. The strength of wood is said to be largely supplied by the middle lamella. Pit membranes are part of the middle lamella. White spruce is a stronger wood than red pine, hence it may be that the middle lamella and pit membranes of red pine are thinner than those of white spruce in the corresponding part of the annual ring.

Runs with white spruce, red pine, cedar and tamarack chips gave no evidence of tori acting as valves which are closed by application of pressure. The pressure-rate curves, Fig. 2, for balsam heartwood, show a slight break at a pressure of 30 or 35 lb. per sq. in. This point requires further study.

Some interesting observations were made in the runs to test the effect of pressure differential. It was noted that, after a constant rate of penetration had been reached (in from 3 to 4 hr.) at a certain pressure, a constant rate was quickly attained on increasing the pressure (except in the case of some runs with balsam). This observation corroborates the conclusion drawn earlier in this section, that magnitude of pressure does not influence the time required to reach an equilibrium condition between water and wood.

**Johnston and Maass observed the same phenomenon in jack pine.*

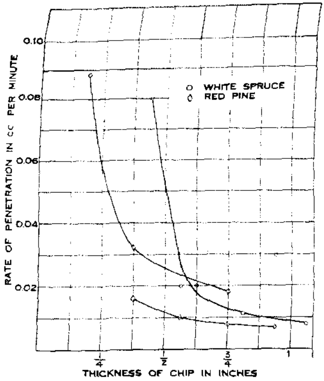

FIG. 3. *Variation with thickness of chip of rate of penetration of water longitudinally through heartwood.*

When a chip is returned from a higher pressure to a pressure at which a constant rate of penetration has already been observed, there is a slight lag in the return of the rate of penetration to the value previously noted. This lag is due to the bulged membranes requiring time to return to the equilibrium position for the reduced pressure. This eventual return to the same value is evidence against the idea of rupturing of pit membranes by pressure.

Influence of Thickness of Chip on Rate of Penetration

The variation in rate of penetration with thickness of chip was investigated for seasoned white spruce and unseasoned red pine heartwoods. The results are listed in Table VII. Results

TABLE VII

VARIATION IN RATE OF PENETRATION WITH THICKNESS OF CHIP

Run no.	Thickness of chip, in.	Rate of penetration in cc./min. with pressure differential		Time in min. for 1 cc. penetration at	
		20 lb./sq. in.	40 lb./sq. in.	20 lb./sq. in.	40 lb./sq. in.
White spruce heartwood					
26	3/16	0.088	0.211	11.4	4.7
27	3/8	0.029	0.069	48.3	14.5
28	3/8	0.035	0.095	28.9	10.5
30	3/4	0.018	0.044	55.5	22.7
34	3/8	0.010	0.037	100	27
35	3/4	0.011		94	
36	9/16	0.0062	0.021	161	48
37	15/16	0.0066	0.024	151	42
38	3/8	0.015	0.037	66	27
42	3/8	0.016	0.038	63	26
39	9/16	0.010	0.029	100	34
40	3/4	0.008	0.022	125	45
41	15/16	0.007	0.018	143	56
Red pine heartwood					
105	7/16	0.08	0.46	12.5	2.2
107	5/8	0.02	0.18	50	5.6
108	9/16	0.02	0.19	50	5.3
109	13/16	0.011	0.06	91	16.6
106	15/16	0.03	0.15	33	6.6
110	11/16	0.008	0.043	125	23

in the third series with white spruce (Runs 38-42) and the series with red pine are shown graphically in Fig. 3. The decrease in rate of flow is due to loss of pressure head. Resistance to flow is encountered in pit membrane pores and, to a lesser extent, in the lumina of tracheids. If the rate of flow were plotted against the probable number of pit membranes that were penetrated, the curve would be flattened out somewhat. The rate of flow does not vary inversely as thickness of chip or as number of pit membranes to be traversed, but decreases more rapidly with increasing thickness. This is due to less bulging of pit membranes and, consequently, less increase in size of pores in the membranes penetrated last, due to loss of pressure head in the pores already penetrated.

Several runs were also made using thin white spruce heartwood chips. These chips ranged in thickness from 1.5 to 4 mm. In the thinner chips (those less than one fibre length, about 3 mm. in thickness) there would be open tracheids through which water could flow without passing through pit membranes. There would also be closed tracheids, where water would have to pass through pit membranes. The data obtained in these runs are given in Table VIII.

TABLE VIII

RATE OF PENETRATION OF WATER THROUGH THIN WHITE SPRUCE HEARTWOOD CHIPS

Run no.	Pressure, lb./sq. in.	Thickness of chip, mm.	Initial rate of penetration, cc./min.	Equilibrium rate of penetration, cc./min.
142	10	1.5	165	
137	10	2.5	23.7	1.00
145	10	2.8	34.6	0.78
139	10	3.4	3.5	0.28
148	10	3.8	1.14	0.18
150	10	3.8	0.85	0.12
143	10	4	1.4	0.07
136	10	5.2	0.2	0.011

The rate of penetration is plotted against thickness of chip in Fig. 4. All the results except that obtained in Run 148 fall on the smooth curve shown. The chip used in Run 148 was faulty so that its effective thickness was really less than that shown. The sharp increase in rate noted when chips less than about 3.75 mm. thick were used is due to the presence of open tracheids in chips of that thickness. This length of 3.75 mm. is therefore about the maximum fibre length of the particular wood. Below this thickness rate of flow would be dependent chiefly on the number of open tracheids present and on the length of these open tracheids. Some flow also takes place through tracheids which have not had both ends cut off. Not more than one pit membrane would have to be penetrated in chips below this length.

The curve in Fig. 4 resembles some of Stamm's curves by means of whic he estimates fibre lengths. Curves of this sort would provide another metho of estimating average fibre lengths.

Effect of Temperature on Rate of Penetration of Water through Heartwood

The variation in rate of penetration of water through a chip at different temperatures was investigated. In two runs (44 and 45) the temperature was maintained at that of the room until a constant rate of penetration was attained. The temperature was then raised to 75° C. and held there for some time and afterwards kept at 125° C. The clamp and chip were then cooled to room temperature and the constant rate of penetration again determined. In another run (46) the rates of penetration alternately at room temperature and at temperatures from 75° to 125° C. were found. Three more runs were carried out first at room temperature, then at an elevated temperature, and lastly at room temperature again. The pressures were 70 lb. per sq. in. driving pressure and 20 lb. per sq. in. back pressure. The data from these six exploratory runs are contained in Table IX. These runs indicated that the chips were being permanently affected in some way at the higher temperatures, so that they did not return to their original permeability when brought back to room temperature. This effect appeared to depend on the temperature employed. Treatment at 75° C. did not alter these chips to any considerable extent, but higher temperatures had a marked effect.

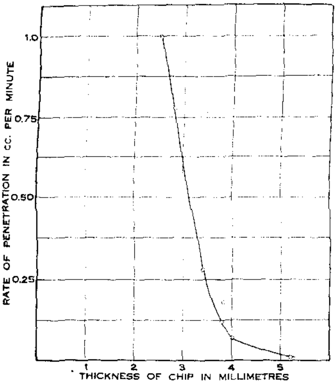

FIG. 4. *Variation in rate of penetration of water through thin chips.*

A series of runs, with similar chips, was carried out to study the permanent effect of elevated temperatures on the chip. By these experiments it was hoped to find at what temperature the permanent effect on the wood became appreciable, and at the same time to study the variation in the rate of penetration with temperature.

The chips used in these runs were ⅜-in. seasoned white spruce heartwood. They were cut from three cylinders taken from adjacent parts of a short block of 18 in. diameter. Before being used they were soaked in water for 24 hr.

The first stage in each run was penetration with water at room temperature, under the same pressures as used in the exploratory runs, until a constant rate was obtained. The temperature of the chip was then raised,

by means of the heating bath, to the higher temperature (50° C. to 140° C.) at which the run was to be carried out. This temperature was maintained for one hour, during which time water penetrated under the same pressures as in the preliminary penetration. At the end of one hour, the bath was removed and the clamp and chip cooled to room temperature. Penetration was continued until a constant rate was again reached. This occurred in about 5 hr. after removal of the hot bath.

At temperatures up to 125° C. a fairly constant rate of penetration persisted during the one hour treatment. At higher temperatures the rate continuously increased during the treatment.

TABLE IX

VARIATION WITH TEMPERATURE OF RATE OF PENETRATION OF WATER THROUGH HEARTWOOD CHIPS

Run no.	Temp., ° C.	Elapsed time at each temp., min.	Rate of penetration, cc. per min.
44	23	320	0.13
	75	60	0.87
	125	90	2.5 to 2.7
	23	130	0.47
45	23	200	0.129
	75	80	0.71
	125	25	4.4
46	23	250	0.138
	75	40	0.57
	23	160	0.146
	100	60	0.83
	23	140	0.158
	125	70	3.7 to 4.0
	23	180	0.31
	125	70	4.7 to 4.9
	23	180	0.52
47	23	200	0.08
	100	250	0.66 to 0.72
	23	130	0.138
48	23	200	0.039
	100	480	0.4
	23	130	0.078
49	23	180	0.040
	75	600	0.20
	23	240	0.052

The results are shown in Table X and the relation between temperature and rate of penetration in Fig. 5. The average initial rate is used as the first point on the temperature-rate curve.

TABLE X

EFFECT OF TEMPERATURE ON RATE OF PENETRATION

Run no.	Rate at normal temp., cc./min.	Temp. of one hour cooking, ° C.	Rate at cooking temp.	Rate after return to normal temp.	Increase in rate
55	0.064	50	0.174	0.059	−0.005
62	0.080	60	0.246	0.087	0.007
67	0.068	70	0.220	0.064	−0.004
61	0.059	71	0.307	0.084	0.025
54	0.052	75	0.415	0.116	0.064
57	0.048	75	0.286	0.077	0.029
56	0.045	80	0.357	0.081	0.036
51	0.074	90	0.678	0.114	0.040
53	0.071	90	0.710	0.098	0.017
50	0.070	100	0.785	0.109	0.039
65	0.083	100	0.660	0.102	0.019
60	0.079	110	1.16	0.134	0.055
63	0.067	115	1.06	0.136	0.059
58	0.065	125	1.06	0.150	0.085
*68	0.172	140	1.8	0.31	0.14
*69	0.132	140	2.6	0.49	0.36

**The chips used in Runs 68 and 69 were not from the same block of wood as the other chips.*

The increase in rate with increase in temperature is due to several factoı The viscosity of water decreases with rise in temperature, and the rate of flo\ according to Poiseuille's law, vari inversely with the viscosity. Wate absorbed at lower temperature taken from the wood owing to th rise in temperature also tends t enlarge the perforations. Also th *flow of hot water through the wood* *may remove in true or colloidal solution some of the material deposited in the pits. Softening of deposited resins and gums at elevated temperatures may permit* greater bulging of pit membranes and hence greater enlargement of their perforations.

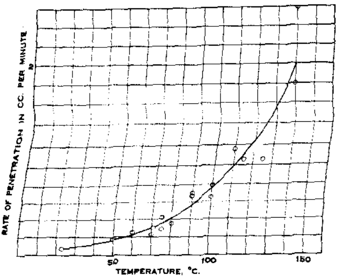

FIG. 5. *Variation with temperature of rate of penetration.*

Fig. 6 shows the permanent increase in rate of penetration due to cooking at the temperatures used. There seems to be little permanent effect of treatment of one hour duration at temperatures below 70° C. Scarth noted that permeability to water was increased to the same extent by boiling wood in water for 12 hr. as by extracting with resin solvents. The permanent effect is probably due to removal of part of the resins and gums encrusting pit membranes and clogging the perforations. Also some rupturing of membranes may take place at the higher temperatures.

Penetrability of Sapwood

Sapwood of most species of conifer has been found to be more amenable than heartwood to impregnation by preservatives. The penetrability of sapwood of five species was investigated during this research. The species studied were white spruce, red pine, balsam, hemlock and tamarack. These species of conifer were found to possess quite different degrees of permeability.

Flow of water was so much more rapid than through heartwood that thicker chips and lower pressures were employed. The data obtained with seven white spruce chips are given n Table XI. Data from runs ısing balsam, red pine, hemlock ınd tamarack sapwood are listed in Table XII.

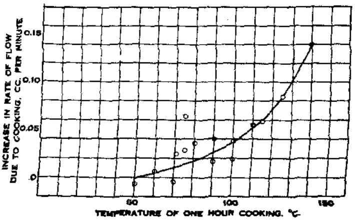

FIG. 6. *Increase in rate of penetration due to cooking one hour at various temperatures.*

TABLE XI

LONGITUDINAL PENETRATION OF WATER THROUGH WHITE SPRUCE SAPWOOD

Moisture condition		Seasoned		Unseasoned					
Run no.		92	98	163	125	126	127	129	144
Thickness of chip, in.		3/8	3/4	3/4	3/4	3/4	3/4	3/4	3/4
Pressure, lb./sq. in.		3	10	1.5	1	1	1	1.5	1.5
Elapsed time		Rate of penetration in cc. per min.							
Hr.	Min.								
	02	0.76	0.09		2.6	8.6	5.7	3.6	8.5
	10		0.05	0.01	2.8	8.7	6.2	4.4	9.7
	20	0.59							
	30	0.51	0.04	0.47		9.5	7.1	4.7	11.4
	35			2.96					
	40			2.82					
1	00	0.43	0.02		4.30	10.7	8.6	5.9	
	30			1.30	4.35	10.8	9.6	6.5	16.4
2	00				4.00		9.9	6.5	16.6
	30						10.0		
3	00						10.1	6.8	
	30								
4	00				3.0			6.4	16.5
5	00				3.0	8.9	8.5	6.0	11.8
6	00				1.6	8.5	8.3	5.5	10.2
7	00				1.2		8.0	4.9	
8	00			0.74	0.9				
24	00			0.53	0.24	4.3			

The seasoned spruce chips behave in the same way as do heartwood chips. The rate of penetration decreases with time, but is much greater than with heartwood of the same thickness under the same pressure. Unseasoned wood, of the species tested, shows an increase in rate of penetration with time, though in some cases, after an initial decrease in rate. A maximum rate of penetration followed by a decrease in rate was observed in the runs listed in Table XII and in the runs with unseasoned chips in Table XI.

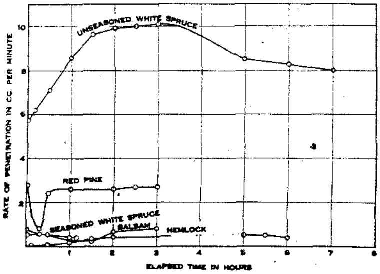

FIG. 7. *Time-rate curves for longitudinal penetration of water through sapwood.*

Time-rate curves showing graphically the variation in rate of penetration with time for one run with each species of wood used (Runs 92, 117, 99, 127 and 164) are plotted in Fig. 7.

TABLE XII

LONGITUDINAL PENETRATION OF WATER THROUGH UNSEASONED SAPWOOD

Species		Balsam		Red pine		Hemlock	Tamarack
Run no.		117	118	99	102	164	167
Thickness of chip, in.		$\frac{3}{4}$	$1\frac{3}{4}$	$\frac{3}{4}$	$1\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{8}$
Elapsed time		Rate of penetration in cc. per min.					
Hr.	Min.	Pressure 1 lb. per sq. in.					
	2	0.53	0.07	2.8	0.25		No appreciable penetration
	7					0.015	
	20	0.59		0.8	0.30	0.048	
	30		0.06	2.4	0.42		
1	00	0.34	0.07	2.6	0.78	0.22	
1	30	0.27			1.7	0.34	
2	00	0.62		2.6	1.9	0.42	
2	30			2.7			
3	00	0.80	0.11	2.7	1.5		
3	30		0.15				
4	00		0.14				
5	00					0.50	
5	30					0.50	
6	00					0.42	
		Pressure 10 lb. per sq. in.					
	2	15.8	8.0		45		
	10	16.2					

Table XIII summarizes a large number of data relating to runs carried out with heartwood and sapwood of various species of wood. Equilibrium rates of penetration are given for heartwood specimens. Maximum rates are given for sapwood specimens for which one was found. In cases where no maximum rate was found, a rate after running some time is given. The only runs presenting a difficulty in the choice of a rate were the runs with seasoned white spruce.

From the rates given in Table XIII it is possible to compare roughly the penetrability of heartwood and sapwood of the different species. Penetration in sapwood of seasoned white spruce is from 50 to 60 times that in the heartwood from the same block. With unseasoned white spruce runs were not carried out at the same pressures for heartwood and sapwood, but by comparison of rates for other species, it is easily calculated that penetration through the sapwood of the log used is about 10,000 times that through heartwood of the same log. Unseasoned balsam sapwood allows about 230 times as much water to pass through as does the same length of heartwood. Unseasoned red pine sapwood is about 600 times as permeable as its heartwood. These ratios are at best rough approximations.

Sapwoods of the species tested differ widely among themselves in penetrability. From these results with unseasoned wood, penetrabilities of hemlock, balsam, red pine and white spruce sapwoods are roughly in the ratios

TABLE XIII

RATES OF LONGITUDINAL PENETRATION IN HEARTWOOD AND SAPWOOD OF THREE SPECIES

Species	Run no.	Thickness of chip, in.	Pressure differential in lb./sq.in.	Rate of penetration, cc./min.
Heartwood				
White spruce seasoned	95	$\frac{3}{4}$	10	0.01
			20	0.027
	(several)	$\frac{3}{8}$	20	0.01-0.06 (Av. 0.03)
	(3 runs)	$\frac{3}{4}$	20	0.008-0.018
White spruce unseasoned	(4 runs)	$\frac{3}{4}$	20	0.006-0.009 (Av. 0.007)
Black spruce unseasoned	(2 runs)	$\frac{3}{4}$	20	0.010
Cedar unseasoned	(3 runs)	$\frac{3}{4}$	20	0.073
Red pine unseasoned	109	$1\frac{3}{8}$	20	0.011
Tamarack unseasoned	(2 runs)	$\frac{3}{4}$	20	0.091
Balsam unseasoned	(6 runs)	$\frac{3}{4}$	10	0.26-0.40 (Av. 0.29)
White pine unseasoned	(1 run)	$\frac{3}{4}$	20	0.98
Sapwood				
White spruce seasoned	98	$\frac{3}{4}$	10	0.024
			20	0.075
	92	$\frac{3}{8}$	10	0.80
White spruce unseasoned	(3 runs)	$\frac{3}{4}$	1	4.35-10.8 (Av. 8.4)
	(8 runs)	$\frac{3}{4}$	1.5	6.8-34.2 (Av. 15.8)
Tamarack unseasoned	167	$\frac{3}{8}$	1.5	No appreciable penetration
Red pine unseasoned	99	$\frac{3}{4}$	1	2.7
	102	$1\frac{3}{4}$	10	45.0
Balsam unseasoned	117	$\frac{3}{4}$	1	0.8
			10	16.0
	118	$\frac{3}{4}$	1	0.15
			10	8.0
Hemlock unseasoned	164	$\frac{3}{4}$	1.5	0.50

of 1: 1.5: 5.5: 17. Heartwoods show relative penetrabilities in order of white spruce, black spruce, cedar, red pine, tamarack and balsam in the ratios of 1: 1.4: 1.5: 10: 13: 40. This relatively great penetrability of balsam bears out the contention of the botanist that in balsam heartwood a considerable proportion of the pit membranes remain in the median position.

Penetration in the Three Structural Directions of Heartwood and Sapwood

The relation between rates of penetration in the longitudinal, radial and tangential directions of both heartwood and sapwood was investigated. Species of wood used were seasoned white spruce, unseasoned red pine and unseasoned white spruce. Water was the penetrating liquid used for most of these runs, but a normal solution of sodium hydroxide was used in some runs with seasoned spruce heartwood. The results obtained are tabulated in Tables XIV, XV and XVI.

The results given as the observed rates of penetration in radial and tangential directions of the wood are, at least in some cases, greater than the actual rates of penetration taking place. The penetrating liquid after enter-

TABLE XIV

VARIATION IN PENETRATION WITH STRUCTURAL DIRECTION IN SEASONED WHITE SPRUCE

Run no.	Direction of penetration	Thickness of chip, in.	Pressure, lb./sq. in.	Rate of penetration, cc./min.	
				At 230° C.	At 60° C.
72	Longitudinal	$\frac{3}{8}$	50	0.075	0.47
71	Tangential	$\frac{3}{8}$	50	0.007	
73	Tangential	$\frac{3}{8}$	50	0.005	0.02
74	Radial	$\frac{3}{8}$	50	0.006	0.018
Sapwood					
92	Longitudinal	$\frac{3}{8}$	20	1.6	
93	Radial	$\frac{3}{8}$	20	0.012	
			50	0.05	
96*	Radial	$\frac{3}{8}$	20	0.016	
			50	0.052	
94	Tangential	$\frac{3}{8}$	20	0.04	
			50	0.09	
97*	Tangential	$\frac{3}{8}$	20	0.008	
			50	0.02	

**The chips used in these runs were coated with pitch around their peripheral surface.*

TABLE XV

VARIATION IN PENETRATION WITH STRUCTURAL DIRECTION IN UNSEASONED SAPWOOD

Species	Run no.	Direction of penetration	Thickness of chip, in.	Pressure, lb./sq. in.	Rate of penetration, cc./min.
Red pine	102	Longitudinal	$1\frac{3}{4}$	10	44.0
	100	Tangential	$\frac{3}{8}$	10	0.41
				20	0.52
				40	0.40
	104	Tangential	$\frac{3}{16}$	20	0.35
	101	Radial	$\frac{3}{8}$	10	0.08
				20	0.11
				40	0.23
White spruce	Av. of 8 runs	Longitudinal	$\frac{3}{4}$	1.5	15.8
	153	Radial	$\frac{3}{8}$	40	0.02
	154	Tangential	$\frac{3}{8}$	40	0.04

TABLE XVI

PENETRATION OF NORMAL SODIUM HYDROXIDE SOLUTION IN THE THREE STRUCTURAL DIRECTIONS OF SEASONED WHITE SPRUCE HEARTWOOD

Run no.	Direction of penetration	Thickness of chip, in.	Pressure in lb./sq. in.	Rate of penetration, cc./min.
78	Longitudinal	$\frac{3}{8}$	50	0.14
80	Longitudinal	$\frac{3}{8}$	50	0.25
79	Radial	$\frac{3}{8}$	50	0.001
76	Tangential	$\frac{3}{8}$	50	0.0012

ing a short distance into the wood, in a direction normal to the longitudinal direction of the tracheids, may then follow the natural path of flow of sap and so come out at the edges of the chip. This liquid entering the enclosed space around the chip, causes a pressure to be registered on the leak gauge. When the pressure around the chip attains sufficient magnitude, liquid may be forced into tracheids near the bottom of the chip and so find its way into the discharge system and appear as liquid which has actually penetrated. In order to obviate this source of error the edges of some chips were coated with hot pitch. This at least caused lower apparent rates of penetration, but the leak gauge still showed that some of the liquid entering was not actually penetrating the chip. Re-entry of liquid into the bottom of chips so treated was probably less than with untreated ones. de Khotinsky cement was used to coat the edges of the chips in the last two runs (153 and 154) of this sort that were carried out. This material formed a more closely adhering layer than did pitch, and re-entry of liquid was probably eliminated in those runs.

Very good evidence that in sapwood, penetration other than longitudinal, is appreciable was afforded in the runs carried out on unseasoned white spruce (11 runs in Table XIII and others in Table XX). In all these runs some liquid appeared in the space surrounding the chip. The pressure used (1.5 lb. per sq. in.) was too small to cause leakage at the contact of clamp and wood. In some of these runs the chips used had a layer of heartwood just outside of the part exposed to liquid. This layer was not ordinarily found to be saturated with water at the end of a run, while the remainder of the outer ring of the chip, being sapwood, was always saturated. This shows the greater resistance of heartwood to lateral penetration. In runs with heartwood, the chips did not show evidence of liquid spreading laterally.

The data in Table XIV show an approximately equal apparent penetration tangentially and radially in white spruce sapwood at both room temperature and 60° C. These rates are about 8% of the rate in the longitudinal direction at room temperature and 4% at 60° C. It must be remembered that this penetration is largely apparent. Very little water actually appeared in the discharge system. The rates are simply for water entering the surface of the chips. The rates shown by sapwood are about 10 times those for heartwood, and considerable water appeared as having penetrated the chips. No definite differentiation between rates in radial and tangential direction was given by these runs. The rates observed in those two directions are on the average slightly over 1% of that in the longitudinal direction.

In the runs listed in Table XV, red pine chips of radial and tangential section were coated with pitch around the edge, while spruce ones were coated with de Khotinsky cement. All these runs showed penetration in the tangential direction to be about double that in the radial direction. Considerable discrepancies are shown in some of these runs. In Run 100, the rate of penetration was about the same at 40 lb. per sq. in. pressure as at 10 lb. per sq. in. The $\frac{3}{16}$-in. chip used in Run 104 showed a lower rate of penetration than the $\frac{3}{8}$-in. one used in Run 100. No longitudinal rates of penetration

with chips of the same thickness, and with the same pressures, are available for comparison with these rates. However, by comparison with the thicker specimens listed, the tangential and longitudinal rates are much less than 1% of that in the longitudinal direction. The relation observed between flow in the three directions in heartwood agrees with the observation of Johnston and Maass that, in heartwood, flow in the axial direction of the fibres is about 100 times that in the other two directions. In the case of sapwood, however, there is a marked difference in the observations, since Johnston and Maass found rates of flow of the same order of magnitude in all three directions. The observation of Johnston and Maass, however, was based on only one determination of rate of flow in each of the lateral directions of the wood.

Table XVI contains some results with white spruce heartwood using normal sodium hydroxide solution as the penetrating liquid. Rates of flow in the radial and tangential directions were found to be practically equal, and less than 1% of that in the longitudinal direction.

The data for rates of flow, in other than the axial direction of the fibres, tabulated in Tables XIV and XVI, are unsatisfactory. The rates observed are probably too high in most cases. In no case would they be too low. Better results are looked for in future runs of this sort, since Runs 153 and 154, using de Khotinsky cement to prevent edge leak, were much more satisfactory than any previous runs.

The theory that penetration is largely a flow from tracheid to tracheid through bordered pit perforations explains the large difference noted between flow in the longitudinal direction and flow in the other directions. The axial dimension of tracheids is about 100 times the other dimensions. Hence, to penetrate the same distance in the radial or tangential direction, water would have to pass through 100 times as many tracheids as in a transverse section. This, of course, would be on condition that communication could be established from tracheid to tracheid to give a continuous path in radial and tangential directions. The structural peculiarity that in most softwoods, pits occur only, or chiefly, on the radial walls of tracheids would favor tangential penetration rather than radial. Also the observation that penetration in heartwood takes place most easily in late summerwood, would cause one to expect easier tangential than radial penetration. Medullary rays probably assist somewhat in radial penetration.

Effect of Intensive Drying on the Rate of Penetration

It has been found that air-dry chips give approximately the same equilibrium rate of penetration as do pre-soaked ones. The suggestion was made that, if wood were intensively dried, it would be impossible to force water through it.

Three white spruce heartwood chips were dried over phosphorus pentoxide *in vacuo* (0.001 mm. of mercury) at 60° C. for three days. These chips were then penetrated at a pressure of 50 lb. per sq. in. with water. Two runs with

the air-dry wood were carried out for purposes of comparison. Results obtained are tabulated in Table XVII.

TABLE XVII

EFFECT OF INTENSIVE DRYING ON PENETRATION

Run no.	Moisture condition of chip	Rate of penetration, cc./min.	
		Initial	Equilibrium
83	Air dry	5.3	0.72
85	Air dry	4.9	0.53
86	Intensely dried	5.5	2.6*
87	Intensely dried	4.9	1.2

**This run was not prolonged to a constant rate.*

The results show no difficulty in penetrating the intensively dried chips. On the contrary, penetration was initially of about the same order of magnitude, but did not decrease to as low an equilibrium rate. This is probably due to some weakening or irreversible change of pit membranes owing to the loss of all their moisture.

Penetration of Sucrose Solutions

Some runs were carried out to test the effect, on the rate of penetration of sucrose solutions, of concentrations ranging from 1.5 to 30% by weight. In the first run (42) of this series, water was run through a ⅜-in. spruce heartwood chip, under a pressure of 40 lb. per sq. in. until a constant rate of penetration was reached. This preliminary penetration was prolonged for nine hours of running time extended over two consecutive days, in order to ensure thorough saturation of the chip and hence a dependable constant rate of penetration. The water being used for penetration was then replaced by a 1.5% sucrose solution, and penetration continued with the same pressure as before, also at room temperature. After four hours of penetration with this solution, one of 3.1% sucrose was substituted. In all, five different solutions, of which the most concentrated was 14%, were used. Water was then run through for nine hours to finish the run.

The data obtained from this run, and from Run 43 with a sucrose solution of 22% concentration are shown in Table XVIII. Similar data from Run 59 which made use of 30% sucrose solution at temperatures from 24° to 100° C. are also contained in the same table. In Run 42, with each solution, the rate of penetration slowly decreased to the rate shown in the table for each concentration, where it remained fairly constant. When water was returned to the apparatus, the rate slowly increased to a value of about one-quarter of the initial rate with water. Run 43 was similar. The rate became constant in less than two hours after starting penetration. One column of Table XVIII shows the product of rate and viscosity of the penetrating liquid. If rate of flow were solely dependent on viscosity this product should be a constant, since rate of flow of liquid through capillary tubes is inversely proportional to the viscosity of the liquid. This was not found to be the case, the product decreasing continuously from that of water to that of the most concentrated solution. It was thought that possibly the effect of sucrose solutions would be to remove absorbed water from the tracheid walls and particularly from pit membranes, thus increasing the size of pores. This

increase in size of pores (if brought about) would permit a greater rate of flow (allowing for viscosity changes). This condition was not found to have been produced.

TABLE XVIII

PENETRATION OF SUCROSE SOLUTIONS INTO HEARTWOOD

Run no.	Temp., °C.	Conc. of solution, %	Elapsed time of penetration, hr.	Rate of penetration, cc./min.	Viscosity of solution, dynes/cm².	Product rate viscosity × 10⁴
42	24	0	7	0.044	0.00919	4.04
		1.53	4	0.020	0.00952	1.90
		3.06	3	0.010	0.00987	0.99
		6.11	3	0.006	0.01071	0.64
		10.00	6	0.0044	0.01211	0.53
		14.00	5	0.003	0.01331	0.39
		0	9	0.011	0.00919	1.01
43	24	0	7	0.038	0.00919	3.51
		22.00	3.5	0.002	0.01565	0.31
		0	3.5	0.010	0.00919	0.92
59	24	30	5.5	0.055		
	125		1	1.06		
	24		6	0.150		
	24		4	0.011		
	52.5		2	0.019		
	75		1	0.034		
	100		1	0.068		

NOTE:—*Thickness of chips, ⅜ in.; pressure, 40 lb. per sq. in.*

Stamm, in an investigation of wood density, found that the non-electrolytes tested, of which one was glucose, were adsorbed to only a very small extent, or even negatively adsorbed by wood.

Effect of Hydrogen Ion Concentration on Rate of Penetration

The effect of normal solutions of sodium hydroxide and of hydrochloric acid on penetration in heartwood and sapwood at room temperature was investigated. In the case of heartwood, seasoned white spruce chips of ⅜-in. thickness were used. These were pre-soaked before being run. Similar chips were used in parallel runs with water, normal sodium hydroxide solution and normal hydrochloric acid solution as penetrating liquids. The results obtained in these runs are tabulated in Table XIX.

Fig. 8 shows time-rate curves for one run using each liquid. The curve with water is of the ordinary type, an equilibrium rate being reached in five hours. The runs with normal sodium hydroxide showed a rate variation quite different from that found with water. The initial rate was less than half that observed with water. The rate of penetration increases to a maximum in about 45 min., then slowly falls off to a constant value higher than that for water. The runs using normal hydrochloric acid show peculiarities of a different variety. The initial penetration in these cases was very rapid.

TABLE XIX

COMPARISON OF RATES OF PENETRATION AT ROOM TEMPERATURE OF WATER, NORMAL HYDROCHLORIC ACID SOLUTION AND NORMAL SODIUM HYDROXIDE SOLUTION THROUGH HEARTWOOD

Run no.	Penetrating liquid	Duration of run, hr.	Rate of penetration, cc./min.	Remarks
68	Water	5	0.172	Time-rate curve in Fig. 8
69	Water	5	0.132	
80	*N* sodium hydroxide	28	0.250	Time-rate curve in Fig. 8
81	*N* hydrochloric acid	26	0.100	Result high due to leak
82	*N* hydrochloric acid	28	0.053	Time-rate curve in Fig. 8
72*	Water	5	0.075	
78*	*N* sodium hydroxide	12	0.14	Time-rate curve similar to Run 80

**The chips used in Runs 72 and 78 were not similar to those used in the other runs listed in this table.*

The rapid rate of flow dropped in about five minutes to a rate which gradually decreased in about four hours to a constant rate lower than that with water.

Data from runs on sapwood using water, normal sodium hydroxide and normal hydrochloric acid solutions, and one of these solutions following water, are tabulated in Table XX. In all cases, where the run was with a single liquid throughout, the rate of flow increased to a value from 25 to 130% higher than the initial rate. The maximum rate was usually observed in from 50 min. to one and one-half hours after the start of the run. The initial rates (where pressure 1·5 lb. per sq. in. was used) for the three liquids showed in general that water flowed through most freely and normal sodium hydroxide most slowly. The initial rate of flow of normal sodium hydroxide solution was only about 20% of that with water. The initial rate with normal hydrochloric acid was somewhat lower than with water.

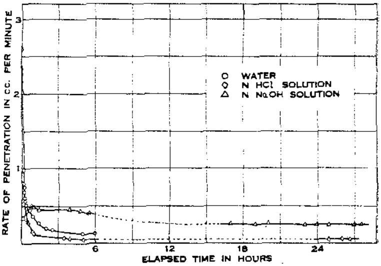

FIG. 8. *Time-rate curves for longitudinal penetration of water, normal sodium hydroxide and normal hydrochloric acid through heartwood.*

The maximum rates with water and normal hydrochloric acid solution were about 35% greater than the initial rates with those liquids. For normal sodium hydroxide the maximum rate, though actually small, was about double the initial rate.

In the runs where water was followed by normal sodium hydroxide solution, the rate of flow of caustic quickly dropped to a rate of about 15% that of

TABLE XX

COMPARATIVE RATES OF PENETRATION OF WATER, NORMAL SODIUM HYDROXIDE AND NORMAL HYDROCHLORIC ACID SOLUTIONS THROUGH WHITE SPRUCE SAPWOOD

Run no.	Penetrating liquid	Rate of penetration, cc./min.		Time to reach maximum rate
		Initial	Maximum	
144	Water	8.5	16.6	1 hr.
129	*N* HCl	3.6	6.5	1 hr. 30 min.
134	*N* HCl	1.0	1.6	3 hr.
135	*N* HCl	20.8	23.2	1 hr.
138	*N* HCl	7.7	13.8	1 hr.
140	*N* NaOH	3.4	6.4	1 hr.
141	*N* NaOH	1.3	2.7	2 hr.
146	Water	4.7	10.9	50 min.
	N NaOH	Rate decreased in 12 min. to 1.4 cc./min.		
	Water	Rate constant at 1.3 cc./min.		
152	Water	11.7	12.6	30 min.
	N NaOH	Rate decreased in 1 hr. to 2.0 cc./min.		
	Water	Rate constant at 1.3 cc./min.		
147	Water	31.0	33.8	30 min.
	N HCl	Rate decreased in 40 min. to 17.2 cc./min.		
	Water	Rate constant at 17.2 cc./min.		
149	Water	12.2	17.8	1 hr.
	N HCl	Rate constant at 16.6 cc./min.		
151	Water	9.0	13.5	40 min.
	N HCl	Rate remained at 13.4 to 13.8 cc./min. for 2 hr.		
	Water	Rate constant at 13.3 cc./min.		

water. With normal hydrochloric acid solution following water, the rate of flow of acid in two cases was only slightly less than that of water, and in the other case, about 50% of the maximum rate of flow of water. The relative viscosity of the liquids must be taken into account. At 25° C. the viscosity of normal sodium hydroxide is 1.236 times that of water, that of normal hydrochloric acid is 1.060 times that of water.

The results in Table XX show the following points: sodium hydroxide, of the concentration used, penetrates heartwood more rapidly than water; hydrochloric acid, after an initial spurt, drops to a rate of flow below that of water; sodium hydroxide shows a rate of penetration lower than that of water in the case of sapwood; hydrochloric acid shows a rate of penetration into sapwood about equal to that found with water; the behavior of caustic soda seems at first glance to be anomalous.

In seeking an explanation of the action of caustic soda solution an experiment was carried out with a thin heartwood chip (1.5 mm.) from the same block as the sapwood used. The average fibre length of this wood is about 3.5 mm. so that over 50% of the tracheids of this chip would be open for flow through them without passing through any pit membrane perforations. This run was carried out with a pressure of 1.5 lb. per sq. in. Water was first forced through until a constant rate was reached, then normal sodium hydroxide until a constant rate for it was reached, after which water was again used as the penetrating liquid. Data from this run are tabulated in Table XXI.

It is a well known fact that sodium hydroxide causes more swelling of wood than does water alone. Also sodium hydroxide is a solvent for lignin. The first result of sodium hydroxide penetration might be some chemical action and solution of pit membrane material. This would have most effect on the thin membrane surrounding perforations. This would account for the initial increase in rate noted (Run 80, Fig. 8). Swelling would also be going on at the same time and would continue after solution had ceased. This swelling, chiefly in pit membranes, accounts for the gradual slowing down of penetration. The final rate with water higher than initially with water, is due to the solvent action. The difference is really higher than the rates indicate, since the viscosity of normal sodium hydroxide at 25° C. is 1.236 times that of water.

TABLE XXI

RATE OF FLOW OF WATER AND NORMAL SODIUM HYDROXIDE THROUGH A THIN HEARTWOOD CHIP (RUN 161)

	Penetrating liquid-water									Penetrating liquid-*N* NaOH								Penetrating liquid-water					
Elapsed time from start of run, min.	2	4	9	15	21	25	34	40	50	53	55	57	66	78	84	116	122	130	145	175	192	210	270
Rate of penetration, cc. per min.	9.8	8.7	7.2	6.4	6.1	6.0	6.0	6.1	6.1	5.3	4.6	4.2	3.0	2.2	2.0	1.8	1.8	1.8	3.0	4.0	4.6	5.1	6.9

NOTE.—*Thickness of chip,* 1.5 *mm.; pressure,* $1\frac{1}{2}$ *lb. per sq. in.; temp., normal.*

With sapwood, the same two actions of sodium hydroxide would take place. Water causes an initial increase in rate of flow through sapwood. The same reason would cause the rate of flow of sodium hydroxide to increase, since the same solvent action around pit membrane perforations would take place. The rate of flow through sapwood is very much greater than through heartwood, hence it is clear that pit membrane perforations must be much larger. Resin ducts must also be taken into consideration. Internal swelling of tracheids takes place, and probably this swelling makes their interior area so small that a considerable resistance to flow is encountered in the tracheids. The inside walls of tracheids, being very largely cellulose, would not be dissolved to as great an extent by sodium hydroxide as would pit membranes. This internal swelling of tracheids may account for the smaller penetration of sodium hydroxide than was noted for water. The data in Table XXI show that with a heartwood chip with open tracheids the behavior of sodium hydroxide is the same as with sapwood. In the thin chip, sodium hydroxide decreased the rate after water had established an equilibrium rate. This could only be due to internal swelling of tracheids, since if only pit membrane perforations were concerned, sodium hydroxide would increase the rate of flow. No resin ducts were present in this thin chip. Viscosity difference must again be taken into consideration. According to these results it would seem that most of the resistance to flow in heartwood is encountered in pit membranes, while in sapwood, resistance in tracheids becomes considerable.

Hydrochloric acid showed a lower rate of penetration through heartwood than did water. This is due to greater swelling, and no solvent action to increase the rate. The rapid initial rate of flow observed is probably due to the absence of absorption of hydrochloric acid, so that swelling is the only action causing a retarding influence, and some little time is required for it to take place. With sapwood, hydrochloric acid seems to have about the same effect as water, the slightly lower rate being due to the greater viscosity.

Miscellaneous Experiments

Three runs were carried out to investigate the flow of nitrogen.

In Run 156 a ¾-in. transverse section of unseasoned white spruce sapwood was used. The chip was quite moist at the start. Five pounds nitrogen pressure was applied to the chip and caused a slow flow of nitrogen through the chip. A pressure of 10 lb. caused a flow about eight times as rapid. Some water was then run in over the chip. The water penetrated, and nitrogen flow followed. This was repeated with 20 lb. pressure with the same result. Water was run through for two hours, after which the pressure was reduced to 6 lb. and nitrogen again let in over the chip. A few bubbles of nitrogen appeared in the discharge, but flow of nitrogen soon ceased. At 10 lb. nitrogen pressures, flow started and increased three fold in 3 min.

In Run 157 a similar chip, of thickness 1½ in., was used. Nitrogen just penetrated at 5 lb. pressure. Pressures up to 35 lb. caused increase in rate of flow.

In Run 158 a 1-in. seasoned white spruce heartwood chip was used. This chip was soaked overnight. Pressures of 20 and 40 lb. caused no flow of nitrogen. Pressure of 60 lb. caused a slow flow for four minutes, after which time flow ceased entirely. After several decreases and increases of pressure and final sudden application of 60 lb. pressure a very slow flow of nitrogen started. Water was next forced through the chip for 11 hr., during which time the rate of flow of water decreased from 0.03 to 0.018 cc. per min. After this water penetration, nitrogen was again forced in over the chip. Pressures from 5 to 60 lb. were tried but no penetration of nitrogen could be obtained.

The general observations from these runs are: (i) with sapwood of the thickness used, nitrogen flows through the green wood under a pressure of 5 lb. or less; (ii) after water has penetrated, slightly higher pressures are required to cause nitrogen penetration; (iii) with the pre-soaked heartwood used a pressure of over 40 lb. was required to cause flow of nitrogen; (iv) after penetration with water, a pressure of 60 lb. was insufficient to cause nitrogen flow; (v) these runs and the observations will be considered in a discussion of the mechanism of flow through wood.

Discussion and Conclusions

Each phase of the subject under consideration has been discussed in conjunction with the experimental work related to it. However, some points require further consideration with reference to the complete results. The

mode of penetration of liquids through heartwood and sapwood requires some discussion. In connection with this, the evidence on action of tori as valves, which can close under pressure and so prevent or impede penetration, requires consideration.

It has been generally recognized by workers on the subject that penetration of liquids through softwoods can take place through tracheids or through resin ducts. However, resin ducts are not present in all woods, while penetration, to a greater or less extent, takes place in all woods. Also, where there are scattered resin ducts, the wood after penetration is found to be wet throughout. This could not take place without the help of agencies other than resin ducts. Water-soluble dyes have been used to follow the path of penetration (16). These, in heartwood, stained chiefly resin ducts and late summerwood tracheids while the remainder of the wood has been found to be wet by water. Also, preservative (22) was found to have slowly spread from late summerwood to the remainder of the annual ring. The wetting by water might take place owing to tracheid flow of water where dye could not penetrate, or it might be due to imbibition of water by the wood substance. Johnston and Maass point out that if imbibition were the means of flow, rate of penetration would be equal in all directions. Imbibition is a more satisfactory explanation of the spread of creosote uniformly. The use of dyes to follow the path of penetration is not entirely satisfactory, since adsorption and absorption of dye molecules by wood substance takes place, and thus interferes with penetration where this takes place to only a small extent. Hence dyes do not necessarily show where all the penetration has taken place.

In sapwood, penetration takes place much too rapidly to be accounted for by the number of resin ducts present.

The rate of penetration through a thin spruce heartwood chip (Run 161) was 6.1 cc. per min. after the chip had become soaked with water. Sapwood chips $\frac{3}{4}$-in. thick under the same pressure showed a rate of flow of from 6.8 to 34.2 (average of 8 runs, 15.8) cc. per min. In penetrating the $\frac{3}{4}$-in. chip several pit membranes would have to be passed through. Volume of flow in open tubes being proportional to length, and about 50% of the tracheids in the thin heartwood chip being open, indicated that little resistance to flow is encountered in pit membranes of sapwood, and that the tracheids themselves provide the greater part of the resistance to flow. In any case, these results would seem to indicate that there is some centripetal thickening of tracheid walls in changing from sapwood to heartwood. The similar behavior of sodium hydroxide in penetrating spruce sapwood and thin heartwood chips also indicates the same thing. Further work on this point is required before drawing definite conclusions.

Action of Tori as Valves

The difficulty encountered in forcing air through unseasoned or pre-soaked wood was attributed by Bailey (3) to surface tension effects when the interface between advancing air and retreating water happened to be in a pit membrane perforation. He thought this resisting force would serve to cause

the torus to be bulged away from the driving force and to close the pit by being pressed against the opposite pit mouth. With a transverse section of wood which had its tracheids filled with water, this effect could be effective in only one pit, or rather, in one series of pits, *i.e.*, most probably the ones leading into the first complete tracheids encountered. But it is quite possible to force gas through sections thicker than one fibre length, *i.e.*, where at least one series of pit membranes must be passed through. Other investigators have used the theory of valve action of tori to explain various phenomena. Thus the closing of pit membranes in the change from sapwood to heartwood has been said to be due to pit membranes being drawn into the lateral position owing to gases on one side and sap on the other. Also it has been said that excessive pressure may decrease penetration by forcing tori into lateral positions, thus closing the pits.

In the present investigation no evidence of tori acting as valves was observed either in heartwood or sapwood. On the other hand some points were observed which disprove the theory in cases where such action might be expected.

In Runs 156, 157 and 158 nitrogen was used in testing the permeability of sapwood and heartwood to gas. With sapwood no great difficulty was encountered, since a pressure of less than 10 lb. per sq. in. was sufficient to cause flow of nitrogen through a $1\frac{1}{2}$-in. chip. With heartwood it was different. A pressure of 60 lb. per sq. in. was sufficient to cause flow for a few minutes through a partly pre-soaked chip (Run 158) but flow soon stopped, but was resumed after several decreases and increases of pressure. This is not the action of a valve. Later a pressure of 60 lb. per sq. in. was insufficient to cause any flow of nitrogen. Gases flow freely, longitudinally, through heartwood in which there is no free water present. Water also flows through under reasonable pressures. Water flows easily after flow has been stopped by application of gas pressure to wet wood. These facts do not point to the presence of anything that can supply valve action.

The surface tension action at membrane perforations does not explain the reason for the large increase in resistance encountered as the thickness of the wood section being penetrated is increased. This can be explained more satisfactorily in conjunction with a manifestation of the force of surface tension in a different way. Each of the tracheids in a series forming a passage through the wood may act as a Jamin's tube, or as part of one formed by intercommunicating tracheids. A Jamin's tube is a capillary tube in which are arranged alternately drops of liquid and bubbles of gas. A pressure exerted at one end of the tube causes adjustment of the menisci between liquid and gas so that each drop of liquid transmits less pressure than it receives. The resistance is proportional to the length of the tube and the number of sequences of drop of liquid and bubble of gas. Such a series of detached drops would be produced on the entry of gas into water-soaked wood. A pressure of 60 lb. per sq. in. was sufficient to initiate a flow of gas. This flow would carry down water from the soaked upper part of the chip to

the interior of the chip where there was no free water, thus lengthening the "Jamin's tube." (The chip was pre-soaked in water without the use of a vacuum.) This increase in resistance might be sufficient to cause cessation of flow, which was actually noted. The resumption of flow noted was due to absorption in the interior of the chip of the droplets formed there, hence again shortening the "Jamin's tube." This cycle could not be repeated after water had penetrated and swollen the chip, since absorption of water would not take place to shorten the Jamin's tube. Internal swelling of tracheids and of pit membranes would also tend to reduce the rate of flow, since in smaller tubes the Jamin effect would be more marked. The low pressure required to force nitrogen through wet sapwood in contrast to the much greater resistance of wet heartwood is due to the larger tracheids in sapwood. This theory fits the observed facts much better than does the one which postulates pit closure by tori.

The rate-pressure curves shown in Fig. 2 show a regular increase in rate with increase in pressure. No discontinuity which could be traced to valve action of tori is noted in these curves. In one run (99) with sapwood the pressure was suddenly increased from 1 to 40 lb. per sq. in. This increase, far from causing a decrease in rate of flow, increased the rate of penetration to 100 times its original value. In several runs, after an equilibrium rate of penetration had been attained, the chip was turned over, or liquid pressure exerted from the opposite end. This change caused no change in the rate of penetration. In another run, after attaining an equilibrium rate of penetration, a back pressure sufficient to prevent any flow was applied for one hour. On removing the back pressure, penetration resumed at the same rate as before. A few chips were re-run after having been air-dried for nearly a year. Rates of penetration noted in two cases were similar to those when first run. Another showed a higher rate.

Photomicrographs supply visual evidence that heartwood differs from sapwood in that most of the pit membranes are sealed in the lateral position. Penetration data tend to corroborate this evidence. Botanists are not agreed as to the cause of this pit closure in the growing tree. It has been shown that closed pits have their tori sealed in the lateral position by actual adhesion, rather than by simple cementation. During this investigation no evidence was obtained to show that pits could be closed by artificial means, in fact any evidence on the subject pointed to the absence of any such closure.

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AN INVESTIGATION OF SEMI-MICRO KJELDAHL METHODS[1]

By J. A. Scarrow[2] and C. F. H. Allen[3]

Abstract

It is shown that the estimation of nitrogen by the Kjeldahl method on a semi-micro scale is more sensitive to variations in procedure than the corresponding macro method.

Several methods of preliminary reduction, recommended for certain types of compounds, have been found difficult to adapt to semi-micro work. A method of reduction, involving use of hydriodic acid and phosphorus, has been developed for many such compounds.

A volumetric method for estimating nitrogen in organic compounds is very desirable. The Kjeldahl method, which is commonly used, works satisfactorily for most simple amines, but is not reliable when applied directly to several classes of compounds (6) such as those containing N-N, N in a ring or polynitro compounds.

In applying the method in this laboratory to 2,4-dinitrophenylhydrazones of various carbonyl compounds, it was found that low results were occasionally obtained with derivatives of known structure. Since these invariably gave correct results by the Dumas method, it seemed probable that the Kjeldahl procedure might be modified so as to adapt it for use with these exceptional hydrazones. A series of experiments was carried out to test the effects obtained from variations in the concentration of the sulphuric acid, potassium sulphate, and glucose. The suggested semi-micro method (1) was found satisfactory except that a few compounds gave better results when more potassium sulphate was used. Since this increased concentration of potassium sulphate did not appear to affect other results, it has been incorporated into the writers' present procedure (Method *A*). Use of catalysts such as selenium, magnesium, and mercury was also tested with no instance of improvement being noted, although these have been shown to be useful in digestion of bulky plant materials (7), where they aid probably by getting rid of the large mass of organic material within a reasonable time. Since simple nitrogen compounds can be digested within 1½-2 hr., provided sufficient potassium sulphate is present, no useful purpose is served by addition of catalysts of this type. Where the nitrogen content runs to 20% or higher, a digestion period of at least 1½ hr. appears to be necessary. Using the concentrations recommended in Method *A*, and heating with a Bunsen burner, samples of animal charcoal were brought to a pale blue in 20–30 min. Such rapid digestion is not advisable since low results invariably follow. Prolonged heating, on the other hand, tends toward slow loss of nitrogen as nitrogen gas (2, 8, 9). This would indicate that this important variable is frequently not defined with sufficient accuracy.

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Preliminary reduction was also tested by a series of runs involving the use of sodium thiosulphate (5), sodium hydrosulphite ($Na_2S_2O_4$) (6), and zinc dust (3, pp. 407-433). None of these gave satisfactory results with hydrazones, and in some cases lower results were obtained than by the use of glucose alone.

It is to be noted that no recent use has been made in this connection of the powerful reducing action of hydriodic acid and phosphorus. A trial run using these gave a comparatively good result; this led to further work being done to devise a method for their utilization. This method, in the writers' opinion, is much superior to others of this type which were tried.

Preliminary Reduction with Hydriodic Acid and Phosphorus

Because of the many possible variations, a large number of runs was necessary.* Some of the results of these are not satisfactory as analyses, but are included as they enable comparisons to be made among different methods. A fairly general method was developed which was satisfactory for all 2,4-dinitrophenylhydrazones and guanidines available and this is given as Method *B*. In all cases investigated this method gave consistently better values than any other involving a preliminary reduction. The procedure in Method *B* was satisfactory for nearly all the compounds analyzed. For a few exceptional compounds it is necessary to vary the period of reduction. Thus *o*—oxyquinoline sulphate was satisfactorily reduced by just bringing the hydriodic acid to a boil over a small shielded flame, and hexamethylenetetramine was best reduced by heating on a steam bath for five minutes. The method failed, however, with dicyanodiamide and with semicarbazide salts. Dicyanodiamide (calcd., 66.6%N) gave yields of 50.0, 54.2 and 52.4% of its nitrogen content when analyzed by Method *A*. This is interesting from the point of view of Werner's formula (10, p. 86), in which this substance is indicated as containing three amino- and three ring-nitrogen atoms.

Method *B* was tested without alteration on several other compounds having nitrogen in a ring, such as cyclonite† which gave a low result, and on indole, carbazol, and mercaptobenzothiazol, all of which gave nearly their theoretical values.

Simple nitrogen compounds, such as amines, amides, oximes, nitro, and dinitro derivatives do not require a preliminary reduction and for these cases Method *A* gives reliable results.

Method *A*

For Simple Nitrogen Compounds

A mixture, made up of 0.02-0.03 gm. of substance, 0.1 gm. of copper sulphate, 7 gm. of potassium sulphate, 2 gm. of glucose, and 15 cc. of concentrated sulphuric acid was digested until colorless, the heat being regulated from time to time when necessary so that this occupied 1½-2 hr. After cooling,

**The present paper is based on results from 150 determinations.*

†The above are given to indicate changes which may be necessary in order to adopt the method to a single difficult compound, such as occurs in control laboratory work.

it was carefully diluted with 150 cc. of distilled water and again cooled in running water. A 24-gm. stick of sodium hydroxide was then added to the flask which was attached immediately to the stillhead, and the ammonia distilled over until violent bumping began. It was found most convenient to weigh the sample on a cigarette paper and put the whole into the flask. Sodium hydroxide, 0.02*N*, and sulphuric acid of about equal strength were found satisfactory. Titration was carried out in presence of alizarin or of methyl red as individually preferred.

Method *B*

For Dinitrophenylhydrazones and Guanidines

A mixture of approximately 0.05 gm. of red phosphorus and 2 cc. of concentrated hydriodic acid (of about 45% strength and usually not iodine-free) was heated to fuming in a 300-cc. Kjeldahl flask held in an upright position on a steambath, the flask being set on a 2–3 in. opening. A 20-gm. sample, wrapped in a cigarette paper was then dropped into the hot liquid. As soon as the paper became disintegrated, a crystal of phenol (0.1–0.2 gm.) was added. The flask was removed from the steambath and immersed in cold water after the sample had been in contact with the hot hydriodic acid for 15–20 min.

A mixture of approximately 0.1 gm. of copper sulphate and 7 gm. of potassium sulphate was then added, followed by 15 cc. of concentrated sulphuric acid, a few drops at a time, and with cooling and shaking. The whole was digested as usual, starting with a low flame for 15 min. and then increasing the heat stepwise so as to complete the digestion in 1½-2 hr. The procedure from this stage was the same as in Method *A*.

Notes

1. The iodine which forms occasionally causes bumping during digestion; this may necessitate addition of a piece of porous tile. The iodine may be recovered as such, or allowed to escape. In any case it must be driven completely out of the flask before the caustic is added. A second burner may be used to remove the iodine in the neck of the flask if necessary.

2. A minimum of wash water should be used to ensure a sharp color change in titration.

3. A result was considered satisfactory if it lay within 0.5% of the theoretical. For purposes of identification, or of ascertaining the number of nitrogen atoms present, a greater deviation is often permissible (6). For many compounds the Dumas* method must be used. Here again however dinitrophenylhydrazones and those compounds having nitrogen in a ring, *e.g.*, antipyrin, require at least double the time necessary to completely burn a simple amine.

**W. M. Lauer and C. J. Sunde (4) have devised a very successful semi-micro modification of the Dumas method, and have used it to analyze several compounds with a maximum deviation of 0.4% from theoretical values.*

TABLE I

COMPARISON OF METHODS

Compound	Nitrogen, %					
	Calcd.	Standard method *A*	Reduction with zinc	Reduction with $Na_2S_2O_4$	Standard method *B*	Dumas method, semi-micro
2, 4-Dinitrophenylhydrazine	28.3	21.8	—	21.0	27.6	28.2
ω-Methoxyacetophenone 2, 4-dinitrophenylhydrazone	16.9	16.4	12.5	14.8	16.8	—

TABLE II

COMPARISON OF METHODS *A* AND *B*

Compound	Nitrogen, %			
	Calcd.	*A*	*B*	Dumas
2, 4-Dinitrophenylhydrazone of:				
Menthone	16.7	16.1	16.4	—
Methyl *i*-hexyl ketone	18.1	15.9	17.6	—
Cyclohexanone	20.0	17.6	20.0	—
γ-Benzoylbutyrolactone	15.1	13.4	15.0	14.8
γ-Chlorobutyrophenone	15.4	13.4	15.1	15.8
n-Butyroin*	17.2	15.6	17.3	17.5
Guanidine hydrochloride	44.0	41.5	43.5	—
Nitroguanidine	49.9	48.5	50.1	—
Diphenylguanidine	20.0	18.9	20.5	—
Carbazol	8.4	7.5	8.2	—
Di-*o*-nitrophenyldisulphide	9.1	4.2	9.1	—
Mercaptobenzothiazole	8.4	8.1	8.6	—
p-Aminoazobenzene	18.0	—	17.5	—
p-Nitrosodimethylaniline	18.6	—	18.8	—
o-Oxyquinoline sulphate†	5.8	1.8	6.1	—
Hexamethylenetetramine§	40.0	37.0	39.2	—

**n-Butyroin 2, 4-dinitrophenylhydrazone gave 14.5 and 14.3% N by the Kjeldahl method (1) when 2 gm. of potassium sulphate was used, whereas method A using 7 gm. gave a somewhat higher value.*

†*Reduced by being brought just to boiling over a small shielded flame.*

§*Reduced by heating on the steam bath for five minutes.*

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COMPETITIVE EFFICIENCY OF WEEDS AND CEREAL CROPS[1]

BY T. K. PAVLYCHENKO[2] AND J. B. HARRINGTON[3]

Abstract

The results of a study of the competing abilities of certain weeds and crop plants are presented. Characteristics studied as possible indicators of competitive efficiency were development of assimilation surface, stomatal number, readiness and uniformity of seed germination and distribution and penetration of root systems.

It is shown that success in competition depends on readiness and uniformity of germination under adverse moisture conditions, the ability to develop a large assimilation surface in the early seedling stage, the possession of a large number of stomata and a root system with a large mass of fibre close to the surface but with its main roots penetrating deeply.

Cereal crops were classified in the order of competing ability as follows:—barley, rye, wheat and oats, flax. *Brassica arvensis* and *Avena fatua* were the most vigorous competitors among the weeds studied.

The farmer has to deal with crops and weeds under many soil and climatic conditions. Both of these groups of plants live in the same environment, and their productive capacities are limited by the moisture, light, nutrients and space available. Each group makes a specific claim upon the productive powers of the field. The competition between them is not very obvious to the casual observer and is therefore easily overlooked. This may explain why competition in cultivated fields has not received due consideration until recently. The struggle is frequently detrimental to crop plants and a weedy harvest results. On the other hand a clean crop may be due to the presence of relatively few weeds, or it may demonstrate the smothering ability of the crop. The latter point is not always appreciated.

According to Clements, Weaver and Hanson (8) the problem of competition in field crops "is centered upon the relative merits of species and varieties in terms of competitive adaptation to seasonal and annual cycles and to the tillage and rotation control of factors and reactions." Each weed and each species of crop has its own merits of competitive adaptation and its own reactions towards its rivals.

For practical purposes the competitive features of both weeds and crops should be studied under definite soil and climatic conditions. Results obtained in investigations along these lines at the University of Saskatchewan from 1930 to 1932 are presented here.

Literature Review

Studies among plant communities and among plants in homogeneous populations have shown that competition is a powerful natural force tending toward limitation or extinction of the weaker competitors. The effects of competition were noted in forest communities by De Crescentiis (10), in the

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plant kingdom generally by De Candolle (9) and in nature as a whole by Charles Darwin. Nägeli (21), 1874, suggested that the probable number of individuals of a competing species in a given habitat is determined by the average life-period and average annual increase.

Early concepts of competition were based on observation of natural forest and grassland communities. In 1903 Clements began systematic research under controlled conditions in an attempt to explain not only the role of competition but also its nature, cause and extent, as well as the parts played by the above and underground parts of the plants. He and his coworkers (4-8) show that "competition is purely a physical process" which "arises from the reaction of one plant upon the physical factors about it and the effect of these modified factors upon its competitor"; that "not only does a small advantage in root, culm or leaf improve the response and hence the chances of the parent plant, but it also encourages the production of tillers, which are potential individuals and constitute what is virtually a reinforcement in the contest"; that "the most important features of the life-forms conditioning the success in competition are, duration or perennuation, rate of growth (top and roots), rate and amount of germination, vigor and hardiness," and that "absorption, transpiration, photosynthesis, chemosynthesis and respiration all affect the physical factors of the habitat directly, producing reactions that determine the course, intensity and outcome of competition." They state also that among the physical factors affecting competition, water is paramount, light next and nutrients third in importance. They point out that "with field crops the problem is centered upon the relative merits of species and varieties in terms of competitive adaptation to seasonal and annual cycles and to the tillage and rotation control of factors and reactions," and that the outcome of competition is equilibrium or dominance of one competitor or extinction of the weaker.

Montgomery (20) studied the competitive abilities of cereal crops. He found, for instance, that Turkey Red wheat is a much stronger competitor than Big Frame wheat. He states that a small admixture of the former in a sample of the latter increased rapidly and in a few years produced 90% of the total stand.

Kiesselbach (19) found that the yield of plants of a given variety fluctuated within broad limits as a result of differences in spacing or surrounding growth. Aaltonen (1) emphasized the importance of the underground parts of field crops in competitive reactions. Sukatschew (22) showed that some biotypes which are vigorous competitors in open cultures do poorly in dense ones.

Brenchley (3, pp. 159-174) in studies of the association of various weeds with cultivated crops found that certain weeds occurred more or less regularly in association with definite field crops, that some weed species were never found with certain crops, and that certain weeds were more or less common among all cultivated crops. She explained this as follows: "One of the chief factors in determining the abundance or scarcity of a particular weed is the degree of competition it is able to withstand successfully, and furthermore, the aboveground struggle for light is as important as the underground struggle for food and water."

Hopkins (13-17) and Barnes and Hopkins (2) have shown by experiments carried on over a period of years at the Dominion Experiment Station, Swift Current, Saskatchewan, that the yield of wheat may be reduced as much as 50% as a result of competition with weeds.

Hanson (12) reports on observations on competition between blue grama and porcupine grasses, on the one hand, and mountain-sage on the other. In the course of several years the mountain sage gained the upper hand and replaced the valuable forage grasses.

Hopper (18) directs attention to the problem of subduing weeds by including in rotations crops which compete vigorously with troublesome weeds. Hanson (11, p. 9) shows that one week's difference in time of cutting alfalfa for hay has a decisive influence on the ability of the following crop to compete successfully with weeds.

From the foregoing review it is obvious that the general significance of the problem has been explained. Investigations have illustrated dominance in admixture, and reactions under field conditions between related plants and other vegetation. Competition between weeds and crop plants presents a useful field of research which, if carried out in great detail, should be of considerable practical significance. Results of practical value can be obtained only by application of a knowledge of the underlying principles coupled with studies of the relative competing efficiencies of weeds and crops.

Scope of Work

It was recognized in this work that there is no such thing as weed-free cultivated land. Field observations carried out for several years showed that every field seeded to a cereal crop supported a mass of seedlings of annual weeds when the crop was about 3 in. high. At harvest a number of these fields bore clean crops. The grain crops had smothered the weeds completely. Other fields of the same or different crops, or seeded at different rates and dates, produced a weedy and scant crop, the weeds having proved more efficient competitors under existing conditions. The determination of the degree of competitive efficiency of each weed and the reasons therefore was the main object of this study.

In competition supremacy may be attained by the species or variety which is able, by virtue of greater physiological activity and morphological adaptability, to utilize the environment most efficiently. Rate of growth may be the best manifestation of such efficiency. Readiness and uniformity of germination, if characteristic of a species or variety, may be of considerable importance. Similarly, facilities for the absorption of water and nutrients and for the assimilation of carbon dioxide may be important factors. These facilities involve all of the plant, including the underground portion. With these points in mind, studies were made of rates of growth, readiness and uniformity of germination, total assimilation surfaces and root development of weed and crop plants.

In 1930 the following weed and cereal plants were studied: weeds:—wild oats *(Avena fatua)*, wild mustard *(Brassica arvensis)*, tumbling mustard *(Sesymbrium altissimum)*, stinkweed *(Thlaspi arvense)*, lamb's-quarters *(Chenopodium album)*, redroot pigweed *(Amaranthus retroflexus)* and wild peppergrass *(Lepidium intermedium)*. Cereals:—Marquis wheat, Prolific spring rye, Hannchen and Sol barley, Banner oats and Crown flax. There were added in 1931, hare's-ear mustard *(Conringia orientalis)*, blue bur *(Lappula echenata)*, wild buckwheat *(Polygonum convolvulus)* and cow cockle *(Vaccaria vulgaris)*; and in 1932 Reward, Reliance and Garnet wheat, Colsess, Regal and Trebi barley and Gopher oats.*

I. Rate of Growth Study

In each of three years (1930-1932) the cereals were grown in competition with the weeds listed for that season. As many as seven trials were used to allow of observation of the behavior of both crop and weed plants under different moisture and temperature conditions. The plots consisted of 8-ft. drill rows, 6 in. apart, seeded at the ordinary rates and depths. The arrangement as shown in Fig. 1 allowed for alternating check and competition plots. Thus there were two checks for comparison with each competition plot. Each competition plot consisted of two rows of the weed with a cereal row between them.

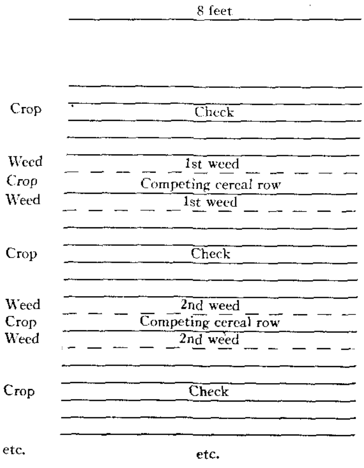


FIG. 1. *Plot layout in competitive study.*

The plants in the four central feet of each check, competing-crop, and weed row were hand pulled at harvest time and cured. The portion of the plant which had been above ground was cut off and used for rate of growth determinations. Data were compiled on weight of seed, weight of straw, total air-dry matter, height and maturity. These were supplemented by notes taken during the growing season on date and percentage of emergence, dates of heading, blooming and maturity and heights at intervals of approximately ten days throughout the season.

**Marquis, Reward, Garnet and Reliance are hard red-seeded; spring sown varieties; Banner is a mid-season, white seeded, open panicle variety; Colsess and Sol are hooded, hulled and six-rowed; Trebi and Regal are awned, hulled and six-rowed*

In order to provide material for comparison a single row of each crop and weed species was grown. These rows were planted 45 in. apart in order to eliminate interspecific competition. In addition single plants were grown each in an area of 100 sq. ft. This allowed the plants complete freedom from competition of any sort. Data from all of this material provided the basis for an estimation of the competitive ability of the different species.

Results

Since the weed and crop plants are so dissimilar in growth habits and seed and other characteristics a fair comparison could hardly be made on the basis of seed or straw yields alone. It seemed logical, however, to use the total dry matter from equal areas for such comparisons. Therefore although the amounts of seed and straw were given due consideration in the interpretation of results, only data on the total dry matter were used in the various comparisons.

The results from the competition plots are expressed in percentage. The total dry matter of each crop was taken from one four-foot row. The yield of the weed grown in competition with a crop is expressed as a percentage of the yield of the crop. This shows at once the degree of success or failure of each competitor (see Table I). The results from the single rows and single plants, on the other hand, are presented in absolute weights, which are reliable criteria of growth where competition was not a factor.

TABLE I

RELATIVE COMPETITIVE EFFICIENCY OF WEEDS AND CEREALS AS SHOWN BY DRY MATTER WEIGHTS OF TOP GROWTH EXPRESSED IN PERCENTAGE OF THE CEREAL YIELDS, 1930-1932

Plants in competition	Hannchen barley (smother crop), %	Prolific spring rye (cleaning crop), %	Marquis wheat (moderate competitor), %	Banner oats (moderate competitor), %	Crown flax (weedy crop), %	Wild oats (weed), %
Cereals	100	100	100	100	100	100
Wild oats	4(2)	10(2)	77(2)	45(4)	35(2)	–
Wild mustard	4(3)	39(2)	43(3)	94(4)	443(3)	286(2)
Tumbling mustard	2(3)	12(3)	12(2)	11(3)	83(3)	34(2)
Hare's-ear mustard	1(2)	8(2)	7(1)	13(2)	386(2)	24(2)
Stinkweed	12(3)	3(3)	–	6(4)	37(3)	6(2)
Peppergrass	1(3)	6(3)	3(2)	1(3)	7(3)	–
Lamb's-quarters	3(3)	36(2)	18(3)	50(4)	374(3)	17(2)
Redroot pigweed	3(3)	21(2)	19(2)	84(3)	442(3)	28(2)
Russian pigweed	–	–	13(2)	73(2)	–	24(2)
Russian thistle	–	–	17(2)	33(2)	–	94(2)
Blue bur	0(2)	1(2)	3(2)	8(2)	85(2)	–
Wild buckwheat	6(2)	4(2)	9(2)	–	421(2)	–
Cow cockle	2(2)	8(2)	20(3)	40(3)	887(2)	25(2)

NOTE.—*All data are averages of material from representative plots grown in one, two or three different years as indicated by the figures in brackets; (4) indicates that in one of the three years the replicates were not uniform enough to be represented by one plot, therefore two plots were used.*

Results on dry matter from competing and non-competing material are shown in Table II. Column 2 shows the dry matter obtained from 4-ft. rows of cereals and from weeds grown between such rows in the competition plots. Column 3 shows the amounts secured from 4-ft. rows of crop and weed plants when rows were widely spaced. In Column 4 appear the amounts from

TABLE II

DRY MATTER FROM TOP GROWTH OF WEED AND CEREAL PLANTS GROWN ALONE AND IN SPACED ROWS IN 1932

Plant	Amount of air-dry matter (gm.) from			
	Four-foot rows		Single plants	
	Competing rows 6 in. apart	5 separate rows 45 in. apart	In competing rows 6 in. apart	Alone on 100 sq. ft.
Marquis wheat	166	427	4.1	28
Reward wheat	185	431	4.3	26
Reliance wheat	216	508	5.1	19
Garnet wheat	150	403	3.2	27
Prolific spring rye	177	389	4.2	18
Hannchen barley	186	631	4.7	43
Colsess barley	176	365	4.3	14
Regal barley	145	594	3.7	39
Trebi barley	146	649	3.8	42
Banner oats	133	502	3.4	33
Gopher oats	158	522	3.9	35
Wild oats (as crop)	76	738	2.0	47
Wild oats (as weed)	44			
Crown flax	41	273	1.0	12
Common wild mustard	34	1223	1.1	228
Tumbling mustard	2	391	0.3	137
Hare's-ear mustard	10	732	0.25	195
Stinkweed	2	411	0.03	21
Peppergrass	1	202	0.01	17
Lamb's-quarters	17	1819	0.5	393
Redroot pigweed	25	1192	0.7	112
Russian pigweed	24	1406	0.6	215
Russian thistle	19	2027	0.5	1662
Blue bur	—	172	—	9
Wild buckwheat	—	378	—	39
Cow cockle	21	1214	0.5	79

NOTE.—*Figures for cereals are averages of ten, and for weeds of four, replicates.*

representative single plants from the competition plots and in Column 5 the amounts from single plants each grown on an area of 100 sq. ft. The data in Column 5 indicate the limits of the natural power of the plant to utilize the environment. This, taken in conjunction with the data in the other columns, gives an idea of the extent of the claims which each species makes upon the environment in competing association. A single striking example of the effects of competition thus illustrated might be quoted here. One plant of Russian thistle growing free from competition produced 1662 gm. of dry matter and hundreds of thousands of seeds, while another, which emerged on the same day but grew in competition with Hannchen barley, produced

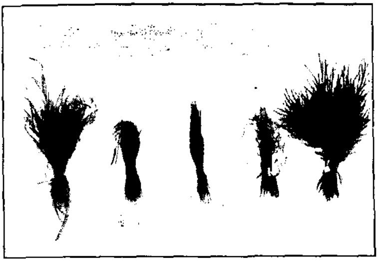


FIG. 2. *Amount of top growth from 4-foot row of hare's-ear mustard grown in competition (from left to right) with (1) Marquis wheat, (2) Prolific spring rye, (3) Banner oats, (4) Hannchen barley, and (5) Crown flax.*

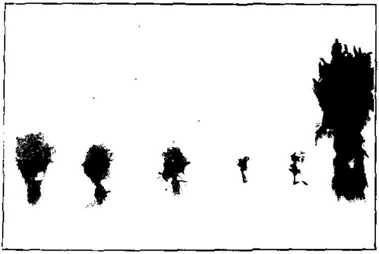


FIG. 3. *Amount of top growth from 4-foot row of redroot pigweed grown in competition (from left to right) with (1) Marquis wheat, (2) Prolific spring rye, (3) Banner oats, (4) Sol barley, (5) Hannchen barley, and (6) Crown flax.*

FIG. 4. *Amount of top growth from 4-foot rows of cow cockle grown in competition (from left to right) with (1) Marquis wheat, (2) Prolifit spring rye, (3) Banner oats, (4) Sol barley (early), (5) Hannchen barley, and (6) Crown flax.*

only 0.5 gm. of dry matter and no seeds. The data in Column 4 indicate the extent of the reduction in vigor of the plants due to competition from the surrounding growth. Similar information may be obtained by comparing Columns 2 and 3 which show the yields from competing rows and from widely spaced rows free from interspecific competition.

Discussion of Results

Throughout the tests differences in the ability of crops to suppress various weeds have been very consistent. This is illustrated in Figs. 2, 3 and 4, which show the reductions in top growth of weeds as affected by six crop varieties.

It was evident from plot observations that the barleys were the most successful competitors and that Prolific spring rye was next in effectiveness. Marquis wheat and Banner oats followed, neither being consistently superior to the other. Crown flax was always the poorest competitor of the crops used, the weeds in competition with it growing profusely and seriously reducing its yield. On the basis of the top growth only, the crops were classified according to their competitive power as follows:

Smother crops:—Hannchen and Sol barley.

Cleaning crop:—Prolific spring rye.

Moderate competitors:—Banner oats and Marquis wheat.

Very poor competitor:—Crown flax.

The weeds studied were also classified according to their competing ability. Where a sufficient number of data were not available classification was based on general observation. Names of weeds classified on this basis appear in brackets. The classification follows.

Serious weeds:—Common wild mustard *(Brassica arvensis)*, wild oats *(Avena fatua)*, stinkweed *(Thlaspi arvense)*.

Serious under certain conditions or in certain crops:—Redroot pigweed *(Amaranthus retroflexus)*, lamb's-quarters *(Chenopodium album)*, cow cockle *(Vaccaria vulgaris)*, tumbling mustard *(Sisymbrium altissimum)*, hare's-ear mustard *(Conringia orientalis)*, wild buckwheat *(Polygonum convolvulus)*, Russian thistle *(Salsola kali)*, Russian pigweed *(Axyris amarantoides)*.

Rarely serious:—blue bur *(Lappula echinata)*.

Not serious:—wild peppergrass *(Lepidium intermedium)*.

The data in Table I largely substantiate the above classification.

The results presented in Table I prove that crop varieties and weed species differ greatly in competitive ability and also that practically all of the weeds suffered greatly from competition with the crop plants. Information of this type should afford a sound basis for the improvement of cropping practices for weed control.

II. Study of the Assimilation Surface

The rate of growth results showed in an empirical way the differences in competing ability among the plants, but did not explain them. It was thought that an explanation might be found in a study of the assimilation facilities of the leaf surface. To this end representative specimens from the widely spaced rows described above were selected at five days after emergence and at the blooming stage, and the total leaf area and numbers of stomata per unit area and per plant were determined.

Methods

The total leaf area was obtained as follows. Specimens were hand pulled and immediately submerged in water to prevent wilting. In the laboratory the leaves of each plant were detached without removing the stem from the water. The entire foliage of each specimen was then withdrawn from the water, hastily mounted over the glass of a blueprint flame, and dried with blotting paper. Blue prints were made immediately. These were cut out and each set of clippings representing the foliage of one plant was weighed. The weights thus obtained were compared with the weight of 100 sq. cm. of blueprint paper. The total leaf area of each specimen was estimated from the formula

$$\frac{Tw \times 100}{cw} = \text{Total leaf area}$$

where *Tw* is the weight of a set of clippings and *cw* is the weight of 100 sq. cm. of blueprint paper.

The figure thus obtained indicated the total assimilation surface for plants with stomata on only one surface of the leaf. In cases where stomata occurred on both sides of the leaves the figure was doubled.

In determining numbers of stomata per unit of leaf area and per plant, camera lucida drawings were made. Ten equal microscopical fields were examined from upper and from lower leaf surfaces in each case. The average numbers of stomata thereon were used as a basis for further estimates. To obtain the average number of stomata the total number was divided by 20 where stomata were present on both leaf surfaces and by 10 where they occurred on only one surface. The numbers of stomata per unit of area vary greatly with the age of the leaf and its position on the plant. Average well-developed leaves were used in all cases in this work.

Discussion of Assimilation Surface Results

Table III includes the data on total assimilation surfaces and on number of stomata of several weeds and grain plants. The data in Column 2 show that wheat with 8.6 sq. cm. at five days after emergence has the least assimilation surface of the four cereal crops studied. Nevertheless this is greater by 5.8 sq. cm. than the greatest assimilation surface among the dicotyledonous weeds studied, that of cow cockle. This would appear to indicate that the

TABLE III

TOTAL ASSIMILATION SURFACE OF WEED AND CEREAL CROP PLANTS FIVE DAYS AFTER EMERGENCE AND AT BLOOMING, 1930

Plants	Five days after emergence			At blooming		
	Total assimilation surface, sq. cm.	No. of stomata		Total assimilation surface, sq. cm.	No. of stomata	
		Per sq. cm.*	Per plant		Per sq. cm.*	Per plant millions
Common wild mustard	2.5	31800	79800	7300	66500	490
Tumbling mustard	—	—	—	2700	19300	52
Hare's-ear mustard	1.1	20900	23000	650	18700	8
Lamb's-quarters	—	—	—	5700	12200	69
Redroot pigweed	.7	13600	9600	1400	29900	42
Russian pigweed	.5	4500	2300	3600	20900	74
Wild sunflower	—	—	—	7600	74300	560
Cow cockle	2.8	13600	39200	2400	—	—
Wild buckwheat	—	—	—	2800	—	—
Marquis wheat	8.6	4700	40300	140	4900	.7
Hannchen barley	18.6	3500	64300	300	4100	1.2
Banner oats	10.2	4200	43200	140	4400	.6
Prolific spring rye	13.1	4900	63700	150	4700	.7
Wild oats	6.4	4700	30300	260	4800	1.2

**The assimilation surface of weeds at this stage is mostly the surface of cotyledons which generally possess less stomata per unit area than normal leaves. This is why in a few cases there are large differences between these data and those given in column 6.*

cereals at this stage are more favorably equipped for assimilation activity than the weeds. But assimilation surface is not the sole indicator of the assimilating ability of a plant. The number of stomata per unit of area is also important. Common wild mustard, which has only 2.5 sq. cm. of assimilation surface as compared with the 8.6 of Marquis wheat, has an average of 31,850 stomata per sq. cm., while Marquis wheat has 4,686.

Since the primary purpose of stomata is to facilitate such physiological activities as transpiration and photosynthesis, it is obvious that these functions are more intense in wild mustard than in Marquis wheat despite the larger assimilation surface of the latter. Undoubtedly the extreme troublesomeness of wild mustard in wheat fields is attributable, at least in part, to this factor. It should be emphasized that any advantage one plant may have over another at this early stage of growth is of extreme importance in the final outcome of competition between them. Fortunately all our cereals, in spite of low stomatal numbers per unit of area, have such large assimilation surfaces at early growth stages that they possess greater numbers of stomata per plant at five days after emergence than any of the dicotyledonous weeds studied, except wild mustard. Thus they are able to develop rapidly their tops and roots and to take more or less complete possession of an area before the weeds attain sufficient development to compete seriously with them. This would account to a large extent for the results presented in Tables I and II.

The data given in Columns 5, 6 and 7 of Table III, on the other hand, show clearly that at the blooming stage all of the weeds possess much larger assimilation surfaces and many more stomata than do the cereals. Therefore a crop which fails to smother the weeds at the early stage cannot do it later. It appears that the noxious characteristics of weeds and the competitive efficiency of cereals are dependent to a large extent on the local assimilation surface and the number of stomata per plant and that, because of the quantitative expression of these characters, the cereals are best fitted to compete with the weeds when both are in the early stages of growth.

III. Readiness and Uniformity of Germination of Weed and Cereal Crop Seeds in Relation to Their Competitive Efficiency

During the years 1930, 1931 and 1932 from 29 to 34 annual weeds and five cereals have been grown side by side in rows. Plantings were made in early spring and approximately every 15 days throughout the growing season in order that the readiness and uniformity of germination of different seeds under different climatic conditions might be observed. In addition, tests were conducted in greenhouse soil boxes, with soil moisture at the optimum for germination and temperature close to 65° F. Readiness of germination is indicated by the number of days from seeding to first emergence, and uniformity by the number of days required by a sample to reach its total germination. Results are shown in Tables IV and V.

TABLE IV

READINESS AND UNIFORMITY OF GERMINATION OF WEED AND CEREAL CROP SEEDS

Plant	Moisture abundant			Moisture scarce		
	No. of days from seed. to first emergence	No. of days from first germination to total	Total germination, %	No. of days from seed. to first emergence	No. of days from first germination to total	Total germination, %
Wild oats	8	14	80	19	22	10
Common wild mustard	4	14	21	11	18	20
Stinkweed	8	17	80	15	20	25
Tumbling mustard	5	14	50	12	19	63
Common false flax	4	10	28	10	15	25
Lamb's-quarters	5	14	80	11	21	24
Russian thistle	4	9	90	10	9	29
Cow cockle	7	13	85	7	14	28
Wild buckwheat	8	17	30	17	23	39
Blue bur	6	14	50	11	18	4
Hannchen barley	5	2	95	6	4	86
Prolific spring rye	5	3	95	7	3	76
Marquis wheat	5	4	90	7	4	69
Banner oats	5	2	95	7	3	58
Crown flax	6	4	43	6	5	32

Each number is the average of 1930, 1931 and 1932 results. In each year there were from four to seven replicates.

TABLE V

READINESS AND UNIFORMITY OF GERMINATION OF WEED AND CEREAL CROP SEEDS UNDER GREENHOUSE CONDITIONS IN 1932

Plant	No. of days from seeding to emergence	No. of days from first germination to total	Total germination, %	Plant	No. of days from seeding to emergence	No. of days from first germination to total	Total germination, %
Wild oats	4	7	74	Wild buckwheat	6	6	11
Common wild mustard	3	8	33	Blue bur	5	7	53
Stinkweed	5.5	6	10	Hannchen barley	3.5	2	98
Tumbling mustard	3	8	69	Prolific spring rye	3	3	84
Common false flax	3	8	1	Marquis wheat	3.5	3.5	100
Lamb's-quarters	4	8	32	Banner oats	3.5	2	98
Russian thistle	2	9	2	Crown flax	3	3	41
Cow cockle	5	6	61				

NOTE.—*Duplicate 100-seed tests under optimum moisture conditions were made in each case.*

Discussion

This study showed that the plants concerned could be grouped into two more or less distinct classes on the basis of readiness and uniformity of germination. Seeds of the first class, which included roughly all of the weed species, showed a low capacity to germinate when soil moisture was scarce.

The cereals however exhibited marked ability to use even very small amount of soil moisture for germination. This fact is of much importance for i indicates that the small grains have a distinct advantage in competitioı with weeds when the soil is low in moisture content at seeding time.

When soil moisture was more plentiful nearly all of the weeds of the mustarc family and most of the pigweeds and cockles germinated as readily as th cereals but never so uniformly. It was only under conditions of extrem drought, or after seeding poorly done, that good seed of the grain crop germinated unevenly. Otherwise, as shown in Tables IV and V a good stanc was established within two or three days after the first plants had emerged Weed seeds, as the same tables show, usually germinated very unevenly.

IV. Root Study of Weed and Cereal Crops in Relation to Their Competitive Efficiency

The greater ability of cereal crop seeds to germinate in the presence of bu slight amounts of soil moisture, and the rapid growth of the plants with the production of large assimilation surfaces in the early stages, are important factors in the competition of cereals with weeds. The root system is the third and perhaps the most important factor. Growth appears above the surface only after the plant has established itself in the soil. It would, therefore, appear that the future well-being of the plant might depend largely on the early development of its root system. The importance of detailed knowledge of the root systems of plants in relation to their competitive reactions can hardly be overemphasized, consequently much emphasis has been placed on root investigation in this study.

The work of three years has shown that the root systems of the weeds and crops differ markedly in growth habit, extent, distribution and penetration. These differences help to explain why some crops grown in weedy districts are relatively clean while others, in areas otherwise relatively weed-free, are badly infested. They also explain why some weeds, although prevalent, never do much harm to crops while others become a menace soon after they invade a district. The results are presented in Tables VI to IX.

The primary purpose of the work was to obtain basic information for an attack on practical weed problems but details of only theoretical interest were not overlooked. The major objective was the determination of the quantitative distribution of the roots in the working areas. For this purpose diagrams indicating the tendency of distribution of roots are not enough; the quantity actually present must be determined. No attempt was made to obtain material by excavation. Blocks of soil large enough to include the root system or at least its complete working sphere were removed and the root material was extricated by washing from the bottom upwards with a fine spray of water from the nozzle of a garden hose. The soil was removed in such fine particles that usually not even the smallest rootlets were appreciably damaged. As the work proceeded the position of primary and secondary roots, and the depth and lateral spread of the main mass of fine rootlets were

recorded on graph paper. The root systems were finally submerged in water and measured. They were then spread, in accordance with the graphical records, dried and photographed. Data presented respecting plants 5 and 21 days old are averages obtained from 5 to 10 individual plants but data on mature plants represent only one or two specimens.

A study was made of the correlation between the mass, length, spread and position of the roots and the rates of growth of the plants. On this basis the weeds and crops were classified as noted in the rate of growth study and in Table VI.

TABLE VI

COMPARATIVE STUDY OF THE ROOT SYSTEMS OF ANNUAL WEEDS AND CEREAL CROPS 5 AND 21 DAYS AFTER EMERGENCE

Weeds	Length in inches		Crops	Length in inches	
	5 days after emergence	21 days after emergence		5 days after emergence	21 days after emergence
Wild oats (serious weed)	34.7	952.9	Hannchen barley (smother crop)	174.5	2286†
Common wild mustard (serious weed)	34.7	4747	Trebi barley (smother crop)	67.5	3974
Stinkweed (serious weed)	17.5	3935	Regal barley (smother crop)	110.1	—
Lamb's-quarters (serious weed in certain crops and under certain conditions)	5.8	3251	Prolific spring rye (cleaning crop)	115.2	2127†
Cow cockle (sometimes serious weed)	27.8	1626†	Colsess barley (cleaning crop)	95.5	—
Hare's-ear mustard (occasionally serious weed)	12.8	1491	Marquis wheat (moderate competitor)	118.5	3047
Redroot pigweed (serious weed in certain crops)	9.0	390.6†	Reward wheat (moderate competitor)	82.6	2550
Russian pigweed (serious weed under certain conditions)	5.1	348.6†	Garnet wheat (more than moderate competitor)	100.8	2773
Tumbling mustard (serious weed in thin stands of grain crops)	7.8	339.8	Reliance wheat (moderate competitor)	98.8	1953
			Gopher oats (more than moderate competitor)	38.1	1367
			Banner oats (moderate competitor)	95.2	875

†*Great care was exercised to procure the complete root system of each plant, if possible. Cases where some minor loss occurred are marked* †.

NOTE.—*All data are averages from five replicates.*

An illustration of the type of material studied is shown in Fig. 11. This single secondary root of Trebi barley with its side branches was separated from the root system at maturity. The branches of this specimen were measured and counted. There were 7,870 branches with a total length of 6,778 in. The accuracy and efficiency of the technique used is demonstrated by the fact that many branches of the first order measuring up to 28 in. still bore their root caps.

Results and Discussion

Table VI includes comparative data for nine annual weeds and eleven standard varieties of cereals. It will be noted that at five days after emergence the root systems of the two most serious weeds, wild oats and common wild mustard, measured only 34.5 linear in. The smallest root system among the cereal crops at this stage measured 38.1 in. Reference to Table III will show that these weeds also had a smaller assimilation surface at the same stage. These facts indicate that there is a high correlation between underground developments and top growth and that the cereals excel the weeds in both these characters at the five-day stage.

A second notable point in Table VI is that at 21 days after emergence common wild mustard produced the greatest root system of any plant studied. It was closely followed by stinkweed, another noxious species. The rest of the weeds fall in approximately the same positions as they were found to occupy in the rate of growth study.

It is apparent that most noxious weeds at 21 days after emergence have greater root systems and greater assimilation surfaces than any of the cereals studied and that consequently no cereal crop can compete successfully with them after this stage of development. It also appears to be true that the noxious characters of weeds are more closely correlated with root development than are the competitive faculties of the cereals. This point will be discussed later.

The relative development of the root systems of weed and crop plants is illustrated in Figs. 5, 6, 7 and 8.

In Table VII root systems of 10 representative plants are analyzed with respect to their numbers of primary and secondary roots and their penetration and distribution in 1931. Hannchen barley had the largest number of

TABLE VII

Analysis of the root systems of wild oats and cereal crops with respect to average number of primary and secondary roots and their penetration and distribution in 1931

Plants	Averages from ten replicates								
	Number of roots per plant		Per cent of roots penetrating deeper than						Length of greatest feeders of the 1st order, in.
			Primary			Secondary			
	Primary	Secondary	18 in.	24 in.	27 in.	18 in.	24 in.	27 in.	
Hannchen barley (smother crop)	7 to 10	7 to 17	80	60	50	20	15	10	13+
Prolific spring rye (cleaning crop)	4 to 5	8 to 14	55	45	35	20	10	5	11.5+
Marquis wheat (moderate competitor)	4	6 to 18	85	75	65	60	55	50	18+
Banner oats (moderate competitor)	3 (rarely 4)	8 to 15	80	70	60	50	30	10	16+
Wild oats (weed)	3	7 to 25	96	80	75	60	40	25	22+

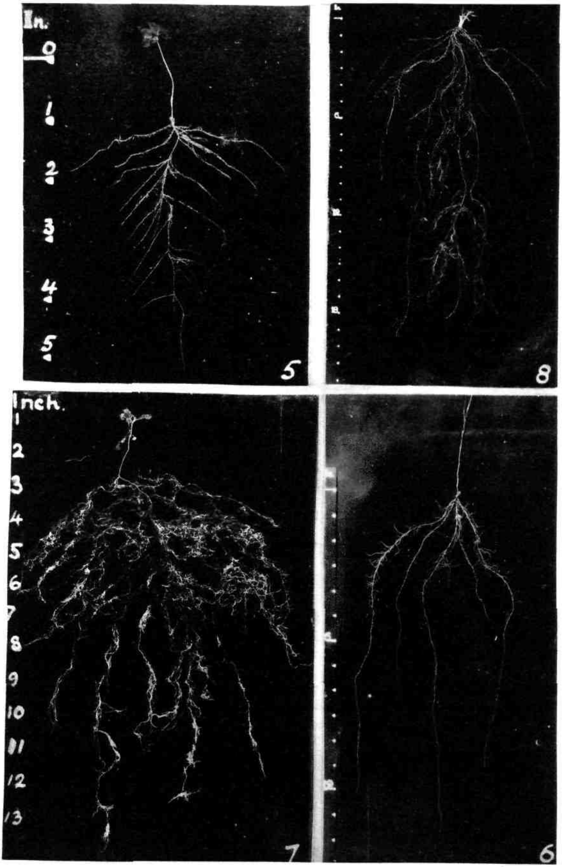
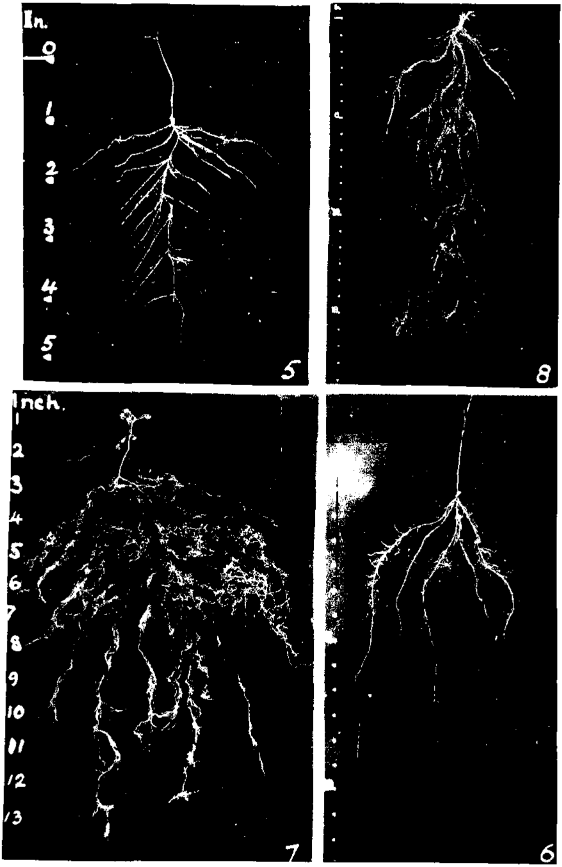


FIG. 5. *A complete root system of common wild mustard at 5 days after emergence, penetrating to a depth of 5 in. and measuring 34.7 in.* FIG. 6. *A complete root system of Garnet wheat at 5 days after emergence, penetrating to a depth of 12 in. and measuring 100.8 in.* FIG. 7. *A complete root system of common wild mustard at 21 days after emergence, penetrating to a depth of 13.4 in. and measuring 4,747 in.* FIG. 8. *A complete root system of Garnet wheat at 21 days after emergence, penetrating to a depth of 24 in. and measuring 2,773 in.*



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primary roots per plant. Prolific spring rye, Marquis wheat, Banner oats and wild oats followed in order. Prolific spring rye had the greatest concentration of primary and secondary roots comparatively close to the surface. Hannchen barley resembled Prolific spring rye most closely in this respect and was followed by Banner oats, Marquis wheat and wild oats. Most of the primary and many of the secondary roots of Banner oats, Marquis wheat and, in particular, wild oats attained much greater depths than did those of Hannchen barley and Prolific spring rye.

The differences just described are important since it seems that the competitive efficiency of each plant, and especially of the cereals, is defined not only by the extent of the root system but by the natural distribution of the roots. For example, under favorable circumstances wheat varieties may develop root systems as great or greater than those of barley (see Table VI). But as these roots are more or less evenly and thinly distributed to a considerable depth (Table VII), wheats are not so successful as competitors with weeds as are rye and barley, the root systems of which are concentrated close to the surface.

Table VIII provides further evidence of close correlation between competing efficiency and development of root systems. Hannchen barley, a smother crop, possessed the greatest root system of the four cereals at all three stages but was excelled at maturity by wild oats. The other cereals followed in the order of their competitive efficiency in the first two stages, but at maturity Prolific spring rye showed divergence from expectation. This may be explained by the fact that the exceedingly fine rootlets of this crop

TABLE VIII

COMPARATIVE LENGTHS OF THE ROOT SYSTEMS OF WILD OATS AND CEREAL CROPS AT THREE DIFFERENT STAGES,* 1931

Plant (ten replicates)	Age, days from emergence	Number of roots per plant, primary	Total average length per plant, in.	Ratio between* 5, 22 and 80 day developments
Hannchen barley	5	6.5	45.7	
(smother crop)	22	7.6	1245.3	3.2 : 89 : 612
	80	8.5	8565.0	
Prolific spring rye	5	4.5	19.1	
(cleaning crop)	22	5.3	682.7	1.3 : 49 : 196
	80	5.3	2748.0	
Marquis wheat	5	3.6	17.7	
(moderate competitor)	22	4.2	649.1	1.3 : 46 : 440
	80	4.0	6160.0	
Banner oats	5	3.1	17.88	
(moderate competitor)	22	3.6	646.6	1.3 : 46 : 468
	80	3.5	6552.0	
Wild oats	5	3.0	13.93	
(weed)	22	3.0	273.0	1.0 : 20 : 721
	80	3.0	10100.4	

***The root system of wild oats five days after emergence is used as a unit for these values.**

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Prolific spring rye	5	4.5	19.1	
(cleaning crop)	22	5.3	682.7	1.3 : 49 : 196
	80	5.3	2748.0	
Marquis wheat	5	3.6	17.7	
(moderate competitor)	22	4.2	649.1	1.3 : 46 : 440
	80	4.0	6160.0	
Banner oats	5	3.1	17.88	
(moderate competitor)	22	3.6	646.6	1.3 : 46 : 468
	80	3.5	6552.0	
Wild oats	5	3.0	13.93	
(weed)	22	3.0	273.0	1.0 : 20 : 721
	80	3.0	10100.4	

**The root system of wild oats five days after emergence is used as a unit for these values.*

break off easily and many may have been lost in washing out the material. Having only three primary roots wild oats started with a smaller root system than any of the cereals and this system increased very slowly at first. Later it developed many more secondary roots than the cereals and at maturity the root system of wild oats excelled that of Hannchen barley.

A good picture of the relative development of the root systems of some crop plants and wild oats is presented in Table IX and Figs. 12, 13, 14, 15 and 16. Table IX shows the lengths and weights and the figures illustrate the distribution of the root systems.

TABLE IX

RELATIVE WEIGHTS AND LENGTHS OF ROOT MATERIAL OF MATURE PLANTS WITHIN BLOCKS OF SOIL 12 BY 12 BY 27 IN. (ORDINARY DRILL ROW PLOTS), IN 1931.

Plants	Air dry material from one-foot row	
	Weights, gm.	Lengths, ft.
Hannchen barley (smother crop)	17.837	20,202
Prolific spring rye (cleaning crop)	7.123	7,783*
Marquis wheat (moderate competitor)	7.181	10,783
Banner oats (moderate competitor)	6.017	5,460
Wild oats (weed)	15.189	20,203

**Rye suffered a heavy loss of fine fibre at the time of washing.*

Fig. 12 shows the concentration of the great mass of root fibre of Hannchen barley in the upper 10 in. of soil. This thorough occupation of the upper stratum leaves little available moisture for weed plants. Some of the primary roots penetrated more deeply in search of moisture but these never went as deep as the roots of rye, oats, or wheat, a fact which may explain the comparative sensitiveness of barley to drought.

Prolific spring rye (Fig. 13) developed less root material per unit volume of soil than did barley, but a proportionately greater amount of it was located immediately below the surface. Very few of its primary, and still fewer of its secondary, roots penetrated to great depths to supply moisture in periods of low precipitation. It is obvious that its competing efficiency is due more to the distribution than to the size of the root system.

In the case of Marquis wheat (Fig. 14) the major part of the root mass is found at a considerable distance from the surface. In the first seven inches there was a fair amount of fine fibre but in the next layer of nine inches there was only a thin network of sparsely branched primary and secondary roots. At still greater depths there was profuse branching of primary and particularly of secondary roots resulting in a denser mass of feeders than was found in the upper strata. Many of the secondary, and even more of the primary roots reached depths of several feet. This type of root system is better adapted for drought resistance than for competition with weeds. While it may account for the suitability of Marquis wheat to the drier zones, it also allows weeds a favorable opportunity to become established because of the scarcity of wheat roots in the upper levels.

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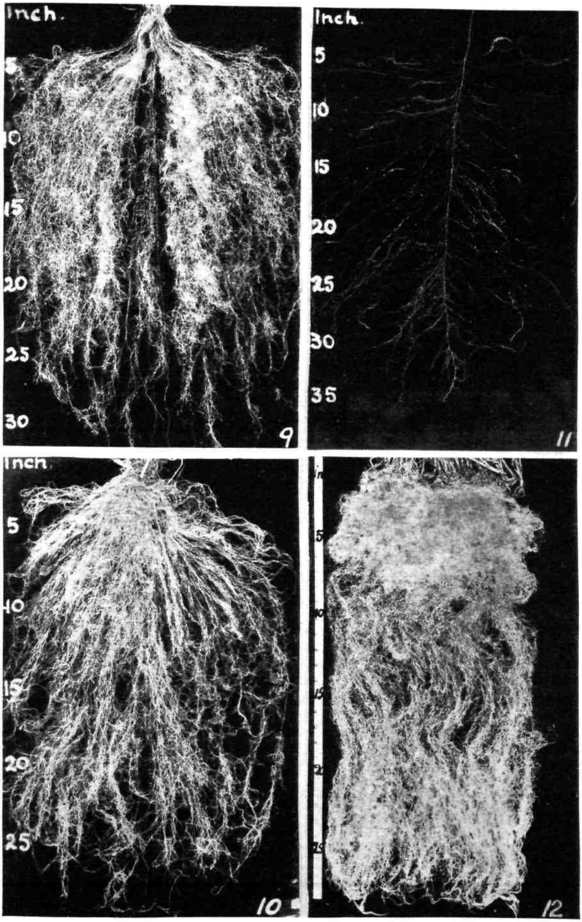

FIG. 9. *Root system of Trebi barley single plant at maturity washed out from a block of soil 26 by 26 by 42 in.* FIG. 10. *Root system of wild oat single plant measuring 7,987 ft.* FIG. 11. *A single secondary root of Trebi barley with its 7,870 branches of 1st, 2nd, and 3rd orders measuring 6,778 in.* FIG. 12. *Root material from one foot of ordinary drill row of Hannchen barley (smother crop) weighing 17.837 gm. and measuring 20,202 ft. (Air-dry material.)*

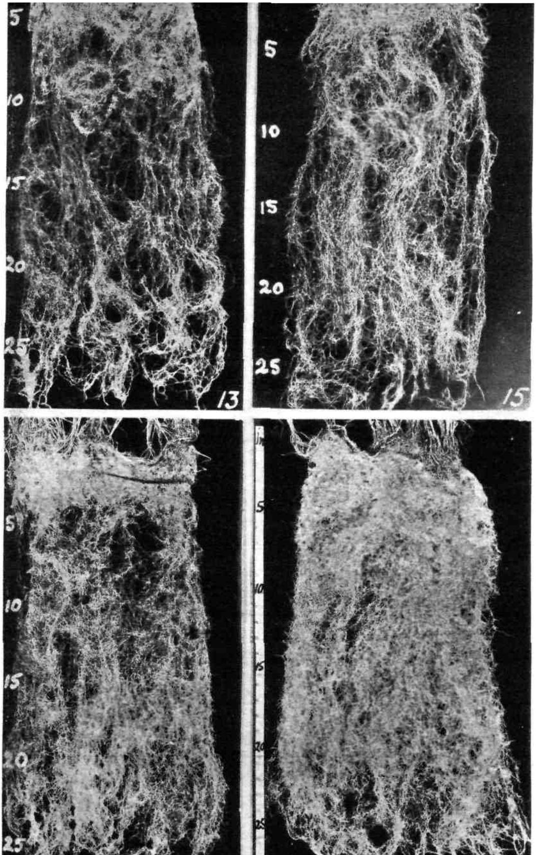

FIG. 13. *Root material from one foot of ordinary drill row of Prolific spring rye (cleaning crop) weighing 7.123 gm. and measuring 7,783 ft. plus a considerable loss in washing. (Air-dry material.)* FIG. 14. *Root material from one foot of ordinary drill row of Marquis wheat (moderate competitor) weighing 7.181 gm. and measuring 10,783 ft. (Air-dry material.)* FIG. 15. *Root material from one foot of ordinary drill row of Banner oats (moderate competitor) weighing 6.017 gm. and measuring 5,460 ft. (Air-dry material.)* FIG. 16. *Root material from one foot of ordinary drill row of wild oats (weed) weighing 15.189 gm. and measuring 20,203 ft.*

The root system of Banner oats (Fig. 15) resembles that of Marquis wheat. This may account for their interchanges of position in the competition studies. Banner oats however has sligbtly more fibre in the upper levels and a more even distribution throughout the occupied depth.

Wild oats (Fig. 16) produced more root material than any of the cereals studied. From the surface to great depths its roots occupied the soil almost completely. Its primary and secondary roots branched and rebranched producing near the surface a dense network which evenly and very gradually diminished with increasing depth. Many side branches of primary and secondary roots were 22 in. or more in length. That cereals are able to compete at all with this weed may be explained first by the slower normal germination of the wild oat and secondly by the fact that the wild oat has not more than three primary roots and develops its root system very slowly at early growth stages.

The mature root systems of Trebi barley and wild oats illustrated in Figs. 9 and 10 provide a basis for comparison of an effective smother crop with a very noxious weed. The total length of the wild oat roots was 7,987 feet and that of the Trebi barley almost as much. A Hannchen barley root system measured 6,917 feet. Such root development evidently accounts both for the effectiveness of barley as a smother crop and for the noxious character of wild oats.

Acknowledgment

The writers wish to acknowledge their appreciation of the time and effort expended on this work by Dr. L. E. Kirk, Dominion Agrostologist, Central Experimental Farm, Ottawa, during 1930 and 1931, when he was in charge of the weed research at the University of Saskatchewan.

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

III. EFFECT OF CROP ROTATION AND CULTURAL PRACTICE ON THE DEVELOPMENT OF FOOT ROT OF WHEAT[1]

By W. C. Broadfoot[2]

Abstract

The following conclusions are drawn from a uniform, co-operative, crop sequence study at seven stations in western Canada, *viz.*, Morden, Indian Head, Swift Current, Scott, Lethbridge, Olds and Vermilion, from 1928 to 1932, inclusive (a total of 28 station-years). Foot-rot damage of wheat is significantly reduced where wheat alternates with summerfallow in a two-year rotation, where it follows summerfallow in other rotations, alternates with oats in a two-year rotation, follows oats in a three-year rotation, follows sweet clover in a three-year rotation, or where wheat is sown late. It is increased where wheat follows wheat, barley, or western rye grass.

Introduction

The loss to the wheat crop in western Canada from that complex, commonly referred to as foot and root rot, is very important. Often it is attributed to one or more of the following organisms:—*Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., *Fusarium* spp., *Leptosphaeria herpotrichoides* de Not., *Wojnowicia graminis* (McAlp.) Sacc. and D. Sacc., and *Pythium* spp. Perhaps only in exceptional cases does one of the fungi mentioned cause all the damage observed. *H. sativum* and several *Fusarium* spp. are constantly associated with foot-rot damage, even when either may not be the principal agent. Some estimates of the loss from the various foot rots in several countries are listed in Table I. As a group these diseases are particularly prevalent and severe in western Canada in favorable seasons, and they are prevalent each year. Undoubtedly the foot rots in Alberta cause a much greater reduction in yield of wheat any season, than do the smuts and the rusts combined.

It was first pointed out by Sanford (53) that the conditions in the black soils of Alberta appeared to be particularly favorable to take-all, and the conditions in the non-black soils distinctly less so. The foot rots caused by *H. sativum* and *Fusarium* spp. develop readily, both in the black and non-black soils of Alberta, Saskatchewan and Manitoba. Moreover, the evidence is that the damage does not diminish, but rather increases with the crop methods used. Bolley (6) attributed part of the increasing unproductiveness of land in North Dakota to the presence of foot-rotting organisms, which

[1] *Manuscript received October 4, 1933.*
Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the Dominion Experimental Farms at Morden in Manitoba, Indian Head, Swift Current and Scott in Saskatchewan, and at Lethbridge in Alberta, and the Alberta Schools of Agriculture at Olds and Vermilion. This paper constitutes one part of a thesis submitted May 3, 1932, to the graduate faculty of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Read at Regina meetings of the Canadian Phytopathological Society, July, 1933. Abstract appears in Proceedings, World's Grain Show and Conference, Ottawa, 1933.
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TABLE I

ESTIMATED LOSS OF THE WHEAT CROP DUE TO VARIOUS FOOT-ROTTING ORGANISMS IN CERTAIN AREAS

Authority	Year	Region	Primary causal organism	Estimated loss
Atanasoff (4)	1920	U.S.A.	*Gibberella saubinetii*	20,000,000 bushels annually. Causes seedling blight as well
Stakman (59)	1919	Minn.	*Helminthosporium sativum*	1 to 50% in individual fields
Heald (26)	1923	Wash.	Foot rot complex	40% of 4,000 acres in Spokane Valley. 30-50% reduction in yield
Christensen (10)	1922	Minn.	*Helminthosporium sativum*	10-20% in Dakota, McLeod and Rice counties
Johnson and Dickson (30)	1919	U.S.A.	*Gibberella saubinetii*	80,000,000 bushels in 1919
Christensen and Stakman (11)	1925	Minn.	Foot rot complex	2-4% of common wheats, 5 to 15% of durum,— as high as 75% in individual fields
MacKinnon (34)	1920	Australia	*Ophiobolus graminis*	12-15% of Australian crop in certain years, with an average reduction for all years of 7%
Stakman (60)	1921	Oregon	*Ophiobolus graminis*	⅓ of wheat crop was lost at Hillsboro
Anon. (2)	1922	Oregon	*Ophiobolus graminis*	Considerable number of fields not worth threshing
Kirby (32)	1925	New York	*Ophiobolus graminis*	0.3 to 2% loss, although losses as high as 20% were observed in individual fields
Sanford (51)	1925	Sask.	*Ophiobolus graminis*	Varied from slight to 15-20% loss in individual fields
Russell (46)	1927	W. Canada	*Ophiobolus graminis*	15-20% loss in individual fields
Sanford (53)	1927	Alta.	*Ophiobolus graminis*	7,000,000 bushels for Alberta only
Sanford (54)	1928	Alta.	*Ophiobolus graminis*	Loss in 30% of 914 fields examined —very significant
Russell (47)	1928	Sask.	*Ophiobolus graminis*	Varied from 1.6 to 18.3% in individual fields
Gordon (20)	1929	Man.	Foot-rot complex	Trace to 100% plants in certain fields.—25% of fields examined showed infection of 50% or more
Doughty *et al.* (13)	1929	England	Foot-rot complex	Loss amounting to 30% of plants
Sanford (12)	1930	Alta.	Foot-rot complex	Found in 271 fields out of 416—2% damage

attack wheat, rather than to a significant loss in fertility of the soil. Why certain micro-organisms should increase or decrease according to soil type, environment, crop or cultural practice, is at present imperfectly understood. However, the work of Sanford and Broadfoot (55), Broadfoot (7, 8), Porter (43), Henry (28) and others suggests that other soil-inhabiting micro-organisms may play an important role in this connection.

Investigations for practical means of combatting the losses from these foot rots have followed two main lines, namely, the securing of naturally resistant varieties, and the devising of crop rotations and cultural practices

suitable to western agriculture. Evidence is that the successful issue of the former method will require a long time, and there is the possibility that varieties may not be obtained which are wholly resistant under all environmental conditions. Hence, it may be necessary to depend more or less on crop rotation and cultural practices for satisfactory control of these diseases.

Although certain investigators in Australia, Europe, the United States of America, and Canada, have recommended various crop rotations and cultural practices to reduce some of the foot rots mentioned, it is felt that, as observations were made under local conditions, and as often only one disease was concerned, such recommendations may not be applicable to the situation in western Canada, or to the foot-rot complex as a whole as it develops naturally in a crop sequence during several years. Consequently, this laboratory initiated, with the co-operation of the Dominion Experimental Stations at Morden, Manitoba; Indian Head, Swift Current, and Scott in Saskatchewan; and at Lethbridge, Alberta, in 1927, and the Alberta Schools of Agriculture at Olds and Vermilion in 1929, an intensive and uniform crop rotation project, where the development of the foot-rot complex might be studied naturally under a wide range of soil and climatic conditions.

Material and Methods

Locations of Project and Soil Type

The locations of the project, soil type, altitude, and approximate average annual precipitation were as follows.—Morden, in S.W. Manitoba; black soil belt, alt. 992 ft., precip. 19 in.: Indian Head in S. Saskatchewan; silty clay loam, alt. 1,927 ft., precip. 17 in.: Swift Current in S.W. Sask.; brown clay loam, alt. 2,432 ft., precip. 15 in.: Scott in W. Central Sask.; brown loam, alt. 2,164 ft., precip. 14 in.: Lethbridge in S. Alberta; light brown silty loam, alt. 2,938 ft., precip. 16 in.: Olds in Central Alta.; black loam, alt. 3,413 ft., precip. 17 in.: Vermilion in E. Central Alta., brown loam, alt. 2,029 ft., precip. 16 in. Precipitation from April to September, inclusive, for the four years, 1928 to 1931, for these seven stations, is shown in Fig. 1.

Outline of Project

The various crop sequences and cultural practices used, each with its reference number, are listed as follows:—

Two-year rotations. 1, Summerfallow, wheat; 2, oats, wheat; 3, barley, wheat; 4, wheat, barley; 5, wheat, wheat; 6, wheat, wheat.

Six-year rotations. 7, Western rye, w. rye, wheat, oats, summerfallow, wheat seeded with w. rye; 8, oats, summerfallow, wheat seeded with w. rye, w. rye, w. rye, wheat; 9, continuous wheat.

Three-year rotations. 10, Wheat, summerfallow, wheat; 11, wheat, summerfallow manured, wheat; 12, summerfallow, summerfallow, wheat; 13, summerfallow, wheat, wheat; 14, summerfallow manured, wheat, wheat: 15, oats, oats, wheat; 16, sweet clover, s. clover, wheat; 17, w. rye, w. rye, wheat; 18, 19 and 20, continuous wheat.

Cultural practices (second wheat crop after summerfallow). 21, Average rate, date and depth of seeding; 22, light rate, average date and depth of seeding; 23, heavy rate, average date and depth of seeding; 24, average rate and date of seeding, but sown deep; 25, average rate and date of seeding, but sown shallow; 26, average rate, date and depth of seeding; 27, average rate, and depth of seeding, but sown late; 28, average rate, and depth of seeding, but sown early; 29, average rate, date, and depth of seeding but

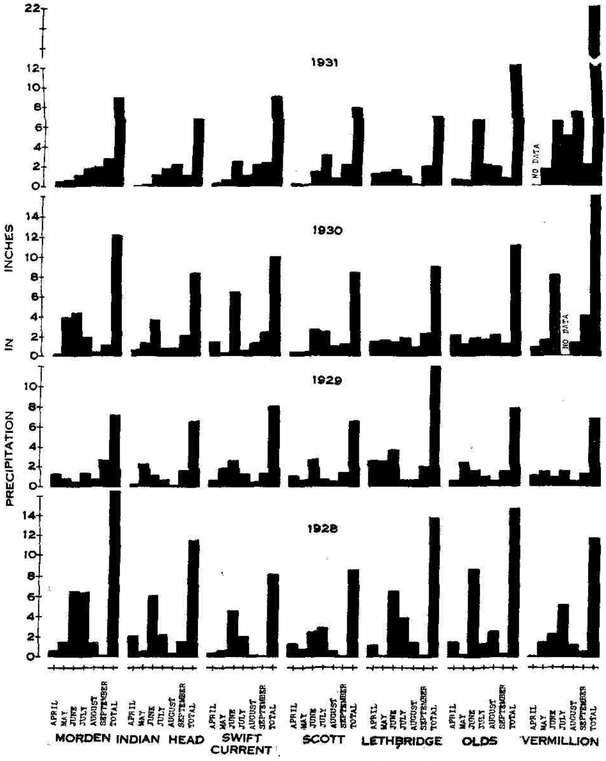

FIG. 1. *Precipitation at seven stations in western Canada during April to September, inclusive, for the four years 1928 to 1932.*

packed "heavy"; 30, average rate, date, and depth of seeding but packed "light"; 31, average rate, date, and depth of seeding not packed.

Cultural practices (first wheat crop after summerfallow). 32, Average rate, date and depth of seeding; 33, light rate, average date and depth of seeding; 34, heavy rate, average date and depth of seeding; 35, average rate, and date of seeding, but sown deep; 36, average rate, and date of seeding, but sown shallow; 37, average rate, date, and depth of seeding; 38, average rate and depth of seeding, but sown late; 39, average rate and depth of seeding, but sown early; 40, average rate, date and depth of seeding, but packed "heavy"; 41, average rate, date and depth of seeding, but packed "light"; 42, average rate, date and depth of seeding, not packed.

The average "rate", "date" and "depth" of seeding means that normally used at a station. "Heavy packing" was twice the weight of the "light packing", where known weights were added to the packer. The manure, where applied, was broadcast at the rate of 12 tons per acre, before the land was plowed for summerfallow. "Early" and "late" seeding means seeding 10 days earlier, and 10 days later, respectively, than the average time for the station. "Heavy" and "light" seeding means one-quarter bushel per acre more or less seed, respectively, than normally used at the station. "Shallow" and "deep" seeding means $\frac{3}{4}$–1 in. below, or above, the average depth for the station.

The results were obtained from four systematically distributed plots, each $\frac{1}{100}$ acre for each unit of the entire experiment. That part of the project dealing with cultural practices, *viz.*, 21–42, was, of necessity, omitted from Morden, Lethbridge, Vermilion and Olds. The plot at Swift Current was appropriated after the third year for an aviation field. Owing to economy measures at Indian Head the project there was discontinued after 1931.

A variety of spring wheat commonly grown in the district represented by the station was used, *viz.* Mindum, a durum wheat, at Morden; Marquis, at Indian Head, Swift Current, Lethbridge and Olds; Ruby, at Scott; and Renfrew, at Vermilion.

Field notes were taken just before the plants were wholly mature. Twenty plants were chosen at random from each of the four replicate plots of a unit, making a total of 80 plants from each unit of the experiment. The crowns and subcrowns were cut longitudinally, and the secondary roots examined and all rated numerically for degree of infection. An adequate representative sample of the basal portion of the plants from each plot was brought to the laboratory and isolations made from them. The results from these isolations are reported in Part IV of this series. The yield was taken at most of the stations.

Methods of Estimating Foot-rot Damage

In America and Europe a uniform method of recording the severity of cereal rusts is in general use, but in the case of foot rots it is sometimes described by the terms "trace", "medium" or "severe". Others indicate it in per-

centage derived from estimated numerical values of arbitrary classes of severity and surviving plants. Any numerical system lends itself well to statistical treatment. McKinney (33) and Christensen (10) have adopted the latter method, each with certain marked modifications, the chief difference being that Christensen uses more classes than McKinney, which would seem one way of reducing the error of personal judgment. Then, too, the method of McKinney would seem better adapted to estimating damage in the seedling stage. With any system, important difficulties arise in determining the actual severity of disease on mature plants grown in the field. For example, the relation between the actual degree of injury to the crown tissue, and the color of this tissue, or its relation to the degree of shrivelling of the grains, is extremely difficult to estimate with any degree of certainty. That is to say, the relation may or may not be sufficiently close. Therefore, it is a question whether the rating should not be entirely based on the condition of the crown and secondary roots. In any case, it would seem highly desirable that a uniform system of recording numerically, in the seedling stage, and also in the mature stage, the severity of the foot rot be officially sanctioned for general use by a recognized body.

Importance of Color

A preliminary examination was made of the relation of the infection rating to discoloration of basal tissue, and also its relation to the general condition of the spike and grains. Material used for the purpose consisted of 200

TABLE II

Degree of infection and color of certain plant parts, and soundness of spikes of plants from wheat after summerfallow and continuous wheat sequences

Plant parts	Degree of infection[1]		Degree of color[2]	
	Wheat after summerfallow	Continuous wheat	Wheat after summerfallow	Continuous wheat
A. Primary roots	3.72 light to medium	6.88 heavy to dead	1.50 brown	2.77 light black
B. Subcrown internode	3.00 light to medium	4.72 medium to heavy	1.23 light brown	2.45 light black
C. Lower half of crown	5.42 medium to heavy	7.04 heavy to dead	1.30 light brown	2.09 brown
D. Upper half of crown	4.88 medium	5.76 heavy	1.27 light brown	2.08 brown
E. 1st set of secondary roots	5.61 medium to heavy	6.99 heavy to dead	1.53 light brown	2.29 brown
F. 1st internode above crown	1.15 trace to light	2.74 light to medium	0.59 very light brown	1.53 brown
G. 2nd set of secondary roots	4.40 medium	5.19 medium to heavy	1.08 light brown	1.59 brown
H. 3rd set of secondary roots (if present)	4.28 medium to heavy	4.79 medium to heavy	0.83 very light brown	1.56 brown
Average*	4.05 medium	5.51 medium to heavy	1.16 light brown	2.05 brown

[1] *Z = 1.88; P = 1193.* [2] *Z = 3.67; P = very great.*

**Soundness of spike:—wheat after summerfallow, 89.8% healthy; continuous wheat, 75.5% healthy.*

plants grown in 1928 from each of two long-time rotations at the Dominion Experimental Station, Lacombe. The first sample was from a rotation in which summerfallow alternated with wheat, and the other sample from continuous wheat. Certain parts of the general foot and root area of these plants (indicated in Table II), were rated separately for color and also for severity of infection. The color was numerically rated as follows: normal 0, light brown 1, brown 2, dark brown 3, light black or dark gray 4, and black 5.

As indicated in Table II, the average color rating of the tissue parts was nearly twice as great on the 200 plants from the sequence wheat after wheat as it was on those from the sequence wheat after summerfallow. When compared on the basis of probable error according to "Student's" method (61), *Z* had a value of 3.67, and the odds were very great that the difference was significant. In the continuous wheat sequence the color of the basal parts ranged from brown to light black, while in the sequence where wheat alternated with summerfallow, it was brown in only one instance, namely, the primary roots, while the other parts were either very light brown or light brown.

The infection rating given the corresponding parts from each of the series mentioned was, as in the case of color, more pronounced for wheat after wheat than for wheat after summerfallow. Based on an average for all parts used, the ratio was approximately 1.3:1, where the *Z* value was 1.88, and the odds 1193:1. The average for color, on a similar basis for the two sequences, was in the ratio of 1.8:1, and with a *Z* value of 367, the odds were very great that the difference was significant.

However, even though there be general agreement in color and infection rating, there are many instances where the degree of color may not correctly indicate the severity of infection. A good example of this is supplied by plant part *D*, upper half of crown, where the color rating for brown was 2.08 in the one case, and 1.27 for light brown in the other, while the infection ratings were 5.76 and 4.88, respectively.

Soundness of Spikes

The general soundness of spikes, including color and condition of kernels, appeared to be even less closely related than the color of the basal part to the infection rating, based on the condition of the crown and root tissue. The ratio of healthy spikes from wheat after summerfallow and from continuous wheat was 1:1.2, while for unhealthy spikes it was 1:2.4. However, the infection rating of the two lots, based wholly on the crown and root tissue, was approximately 1:1.3. Thus, in spite of the fact that certain cases arise where the relation might be fairly close, the actual condition of the crown and root tissue is not always reflected by the condition of the spike. The same may be said regarding yield.

Consequently, in this experiment the infection rating was based wholly on the condition of the crown and secondary roots. The primary roots were ignored, since on mature plants in the field these may be an uncertain quantity. The various degrees of infection were rated as follows:—clean 0, trace 1,

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TABLE III

EFFECT OF CROP ROTATION AND CULTURAL PRACTICE ON THE DEVELOPMENT OF FOOT ROT OF WHEAT AT SEVEN STATIONS IN WESTERN CANADA DURING 1928 TO 1932, INCLUSIVE. AVERAGE YEARLY RESULTS ARE GIVEN

	Infection rating							Number of fertile culms					Number of total culms							Yield						
A*	1928	1929	1930	1931	1932	Av.	B†	1930	1931	1932	Av.	B	1928	1929	1930	1931	1932	Av.	B	1928	1929	1930	1931	1932	Av.	B
2-year rotations																										
1	1.0	2.8	2.5	1.3	1.9	1.9	28	1.6	1.6	1.8	1.7	18	2.0	1.5	2.0	2.8	2.8	2.2	28	24.8	16.5	32.2	35.4	37.0	29.2	21
2	0.5	2.1	2.7	2.1	2.4	2.0	27	1.7	1.3	1.4	1.5	17	1.9	1.3	1.9	2.0	2.3	1.9	27	24.5	9.4	26.2	24.6	33.5	23.6	21
3	0.6	2.4	4.7	4.5	5.5	3.5	27	1.4	1.3	1.3	1.3	17	1.7	1.2	1.8	2.1	2.2	1.8	27	22.4	10.5	25.3	24.6	33.1	23.2	21
4	0.6	2.3	6.1	5.7	6.6	4.3	25	1.5	1.3	1.3	1.4	17	1.6	1.3	1.9	2.1	2.3	1.8	25	25.8	12.6	28.0	41.5	47.7	31.1	20
5	0.6	2.5	5.1	4.7	5.1	3.6	27	1.4	1.4	1.3	1.4	17	1.8	1.3	1.7	2.2	2.2	1.8	27	24.2	9.4	23.9	23.4	32.1	22.6	21
6	0.8	2.4	5.2	4.9	5.3	3.7	27	1.4	1.3	1.3	1.3	17	2.0	1.3	1.8	2.1	2.3	1.9	27	23.3	8.8	25.7	25.9	28.2	22.4	21
6-year rotations																										
7	1.8	4.4	3.7	2.0	2.2	2.8	25	1.7	1.7	1.6	1.7	17	2.1	1.5	2.2	2.6	2.6	2.2	25	34.6	15.7	39.3	31.0	34.6	31.0	18
8	1.5	3.1	5.2	4.8	5.3	4.0	24	1.5	1.4	1.4	1.4	16	1.7	1.4	1.9	2.2	2.3	1.9	24	33.1	12.9	25.3	27.6	24.4	24.7	18
9	0.1	5.0	5.3	4.6	5.4	4.1	11	1.4	1.6	1.3	1.4	9	2.7	2.2	1.7	2.3	2.2	2.2	11		8.5	41.1	56.9	37.8	36.1	6
3-year rotations																										
10	1.1	2.6	2.3	1.4	2.1	1.9	28	1.7	1.9	1.7	1.8	18	2.4	1.4	2.2	2.8	2.6	2.3	28	25.4	15.2	36.0	35.2	31.6	28.7	21
11	1.4	3.3	2.6	1.6	2.3	2.2	25	1.7	2.0	1.9	1.9	17	2.0	1.5	2.2	3.1	2.8	2.3	25	29.1	20.2	42.3	36.2	34.3	32.4	18
12	1.5	2.4	2.0	1.4	2.0	1.9	27	1.6	1.9	1.8	1.8	18	2.0	1.6	2.2	2.8	2.7	2.3	27	26.0	17.4	46.5	38.2	35.6	32.7	20
13	1.0	2.4	4.8	4.2	5.0	3.5	26	1.4	1.5	1.3	1.4	17	1.9	1.5	1.9	2.4	2.3	2.0	26	20.5	9.8	31.2	27.2	27.6	23.3	20
14	0.7	3.1	4.6	4.3	4.5	3.4	23	1.5	1.5	1.4	1.5	16	1.7	1.3	2.0	2.4	2.4	2.0	23	23.7	11.4	35.8	27.0	30.7	25.7	17
15	1.0	2.3	2.3	2.2	2.3	2.0	26	1.5	1.5	1.4	1.5	17	2.2	1.4	1.8	2.3	2.3	2.0	26	24.3	9.8	29.6	26.5	28.0	23.6	20
16	0.9	2.8	2.3	2.2	2.6	2.2	27	1.5	1.7	1.7	1.6	18	2.0	1.5	2.0	2.7	2.7	2.2	27	24.8	10.7	35.3	32.5	33.0	27.3	20
17	0.8	2.4	4.6	4.4	5.3	3.5	27	1.4	1.5	1.3	1.4	18	2.1	1.6	1.9	2.5	2.3	2.1	27	19.8	12.3	26.7	28.7	28.4	23.2	20
18	0.0	3.4	5.3	5.1	5.2	3.8	11	1.5	1.5	1.3	1.4	9	2.1	2.4	1.9	2.5	2.3	2.2	11		15.3	42.9	57.7	31.8	36.9	6
19	0.0	4.0	5.6	5.1	5.0	3.9	11	1.5	1.5	1.4	1.5	9	2.7	1.9	1.8	2.6	2.3	2.3	11		14.2	47.9	55.2	29.8	36.8	6
20	0.0	3.8	5.4	4.5	5.2	3.8	11	1.3	1.4	1.2	1.3	9	2.8	2.3	1.8	2.4	2.1	2.3	11		18.7	38.4	49.0	33.1	34.8	6
Cultural practices (Summerfallow, wheat, wheat)																										
21	0.9	1.2	4.5	2.9	5.2	2.9	11	1.3	1.1	2.0	1.5	5	1.6	1.1	1.9	2.0	3.0	1.9	11	20.2	4.2	16.2	13.8	19.0	14.7	11
22	0.8	1.0	4.4	3.0	5.2	2.9	11	1.4	1.5	1.8	1.6	5	1.7	1.2	1.9	2.4	2.8	2.0	11	18.9	4.9	16.0	15.9	19.2	15.0	11
23	0.7	1.4	4.2	3.2	5.2	2.9	11	1.1	1.2	1.7	1.3	5	1.5	1.1	1.4	1.9	2.7	1.7	11	21.3	4.0	16.9	14.7	17.8	14.9	11
24	1.0	1.9	4.1	3.2	5.7	3.2	11	1.4	1.3	1.5	1.4	5	1.9	1.1	1.9	2.2	2.5	1.9	11	19.6	4.3	18.4	13.1	18.3	14.7	11
25	0.9	1.4	4.2	3.1	5.7	3.1	11	1.4	1.2	1.8	1.5	5	1.8	1.1	1.8	2.1	2.8	1.9	11	17.8	4.8	18.7	12.4	17.7	14.3	11
26	0.8	1.0	4.3	3.1	5.6	3.0	11	1.3	1.3	1.6	1.4	5	1.9	1.1	1.8	2.2	2.6	1.9	11	19.6	4.1	18.6	14.2	19.1	15.1	11
27	0.7	2.2	2.4	2.1	2.5	2.0	20	1.7	1.7	1.9	1.8	13	1.3	1.1	2.1	2.4	2.9	2.0	20	16.9	4.9	19.3	16.8	19.2	15.4	11
28	1.2	2.1	4.8	4.7	5.3	3.6	20	1.5	1.6	1.5	1.5	13	2.1	1.2	1.9	2.4	2.5	2.0	20	17.0	3.4	16.5	11.5	18.8	13.4	11
29	1.5	1.9	5.0	4.1	5.4	3.6	20	1.3	1.6	1.4	1.4	13	1.8	1.4	1.7	2.5	2.4	2.0	20	21.5	3.8	19.0	15.4	16.3	15.2	11

light– 2, light 3, light+ 4, medium– 5, medium 6, medium+ 7, heavy– 8, heavy 9, heavy+ 10. These data were further supplemented by the total number of culms, number of fertile culms, and yield.

Experimental Results

For convenience, the summarized results of each crop sequence or cultural practice, for all the stations (totalling 28 station-years) will be discussed separately. The data taken at every station from 1928 to 1932, inclusive, on infection rating, total number of culms, number of fertile culms, and yield of wheat, are compiled in various tables*, and, as it is impossible to present these in detail here, only average results will be used in this report. However, it is deemed advisable to present in Table III the average results for each year.

The agreement in results among replicates of a given treatment at the various stations during one year was reasonably satisfactory, under the conditions of the experiment. An example of the variation among the four replicates of each of the two continuous wheat series at the various stations, in 1930, with Z and P values calculated as before by Student's method, is supplied in Table IV. Satisfactory explanation for the large variation at Vermilion is not available. However, in no other year did such an unusual variation occur, either in the replicates of these, or other sequences at any station. It may also be mentioned that the variation probably would be less in the continuous wheat sequence than it would be where wheat followed different crops. Naturally the wide range of soil and climatic conditions

TABLE IV

INFECTION INDEX FOR THE REPLICATES, MEANS, AND Z AND P VALUES CALCULATED BY "STUDENT'S" METHOD FOR TWO CONTINUOUS WHEAT SEQUENCES AT SEVEN STATIONS

Station	Plot no.	Infection index					Values for	
		Replicate						
		A	B	C	D	Av.	Z	P
Morden	5	4.7	3.4	3.7	4.5	4.1	.02	1.02
	6	3.7	2.8	4.2	5.1	4.0		
Indian Head	5	5.4	5.5	6.1	6.9	6.0	.69	5.29
	6	5.7	5.4	5.4	5.9	5.6		
Swift Current	5	6.5	6.0	5.9	7.3	6.7	.05	1.12
	6	6.7	6.0	6.8	6.0	6.4		
Scott	5	4.2	4.3	3.2	4.9	4.2	.32	2.24
	6	4.3	4.9	3.7	4.3	4.3		
Lethbridge	5	5.3	5.5	5.9	5.3	5.5	.10	1.29
	6	5.9	5.9	5.8	4.6	5.6		
Olds	5	4.4	4.9	4.1	4.2	4.4	.10	1.29
	6	4.8	4.0	5.2	4.4	4.6		
Vermilion	5	4.7	4.6	4.6	3.9	4.5	2.62	102.0
	6	6.0	6.5	4.7	5.1	5.0		

*The results for each crop sequence and station for the years 1928 to 1932, inclusive, are on file and are available at the Dominion Laboratory of Plant Pathology, Edmonton, Alberta.

which existed between one station and another in a given year, tended to affect the infection rating, yield and other data from all treatments. But the significance of these climatic and soil factors cannot be discussed fully at this time. Suffice it to say, such variations would not seem to affect the conclusions in this paper.

Crop Sequence

Continuous wheat. Guyot (25), Gram (21), Gram, Jørgensen and Rostrup (23), and Tedin (62) in Europe, and Russell (48), and Sanford (52) in Canada, have pointed out that wheat suffers most from foot rot where this crop is grown continuously on the same soil. The average results for the continuous wheat sequence, treatments Nos. 5 and 6 (Table V), for the years 1928 to 1932 inclusive, indicate that this sequence gave the highest infection rating and the lowest yield and number of fertile culms of any in the entire experiment.

TABLE V.

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
5	Continuous wheat	3.7	1.4	1.9	20.4
6	Continuous wheat	3.9	1.4	1.9	20.7

NOTE:—*I.R., F.C., T.C., and Y.B., indicate infection rating, fertile culms, total culms, and yield in bushels per acre, respectively. These abbreviations will be used subsequently in tabular listings.*

Summerfallow alternating with wheat. Gram and Rostrup (22), and Petersen (41) in Europe, and Fraser, Simmonds and Russell (18), and Sanford (52) in Canada state that take-all is less severe where wheat follows summerfallow than where it follows wheat. This is amply verified throughout this experiment. In fact, wheat grown after summerfallow was much less severely attacked than it was after any other crop or practice, the infection rating being 1.9 for the former, and 3.7 for continuous wheat sequence (treatments Nos. 1 and 5). Also, the average total number of culms, average number of fertile culms, and yield were uniformly greater where wheat followed summerfallow than where it followed wheat. This is indicated in Table VI.

TABLE VI

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
1	Summerfallow alternating with wheat	1.9	1.7	2.2	27.1
5	Continuous wheat	3.7	1.4	1.9	20.4

Barley alternating with wheat. Gram, Jørgensen and Rostrup (23), Moritz (36), and Heitz (27) in Europe, and Russell (48) in Canada, state that where wheat followed barley, the development of take-all was favored, although Petersen (41) in Sweden suggests that this disease can be practically eliminated by a crop of summer barley. Also, it is maintained that injury from *H. sativum* is frequently increased when wheat is grown after barley.

In the rotations described here the average infection rating on wheat grown after barley was 3.5, which is almost as great as it was on wheat grown after wheat, *viz.* 3.7 (treatments Nos. 3 and 5). The difference in average total number of culms, average number of fertile culms, and yield was very slight for the two sequences, being indicated by the results in Table VII.

TABLE VII

Treat-ment	Rotation	I.R.	F.C.	T.C.	Y.B.
3	Barley alternating with wheat	3.6	1.4	1.8	21.1
5	Continuous wheat	3.7	1.4	1.9	20.4

Oats alternating with wheat. Heitz (27), Petersen (41), Sanford (52), Russell (48), and Simmonds (58) state that foot rot is greatly reduced where wheat follows oats. Richardson (44), Schaffnit (56), Kirby (32), Fraser, Simmonds and Russell (18), Wolf and Lehman (64), and Russell (47), state that in general oats are highly resistant or immune to *O. graminis*. However, Jones (31), in Wales, described the formation of perithecia of this parasite on oats, and, in Germany (36), Australia (1), and Denmark (3) certain varieties were found susceptible.

The infection rating on wheat after oats shows quite conclusively that this crop greatly reduces foot-rot injury, and in this respect is fairly comparable to the effect of summerfallow. For example, the infection rating was 2.0, while for continuous wheat it was 3.7. In Table VIII is a comparison of averages of infection rating, number of fertile culms, number of total culms, and the yield, from this sequence, with the continuous wheat sequence (treatments Nos. 2 and 5).

TABLE VIII

Treat-ment	Rotation	I.R.	F.C.	T.C.	Y.B.
2	Oats alternating with wheat	2.0	1.5	1.9	21.5
5	Continuous wheat	3.7	1.4	1.9	20.4

Two crops of oats followed by wheat. The effect of two crops of oats on the infection rating was apparently identical with that of one crop of oats. The results from this sequence are compared in Table IX with those from the continuous wheat sequence, and also with those from one crop of oats (treatments Nos. 15, 2 and 5).

TABLE IX

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
15	Wheat after two crops of oats	2.1	1.4	2.0	21.8
2	Oats alternating with wheat	2.0	1.5	1.9	21.5
5	Continuous wheat	3.7	1.4	1.9	20.4

Two crops of sweet clover followed by wheat. The average infection rating in this sequence was very similar to that in the sequence in which summerfallow alternated with wheat (treatments Nos. 16 and 1). The averages of the total number of culms and number of fertile culms were increased slightly, and the yield, which depends upon the catch of sweet clover secured, was on the whole improved (Table X).

TABLE X

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
16	Wheat after two years of sweet clover	2.1	1.7	2.3	25.4
1	Wheat after summerfallow	1.9	1.7	2.2	27.1
5	Continuous wheat	3.7	1.4	1.9	20.4

Two crops of western rye followed by wheat. Samuel (50), Russell (48), Padwick and Henry (38), and others have reported that take-all is favored when wheat follows western rye. Also, some observers contend that damage from either *H. sativum* or *Fusarium* spp. is increased by this crop. The infection rating from this sequence was about as high as it was from the continuous wheat sequence or from the sequence wheat after barley (treatments Nos. 17, 3 and 5). The comparison is shown in Table XI.

TABLE XI

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
17	Wheat after two crops of western rye	3.9	1.4	2.1	22.2
3	Wheat after barley	3.5	1.4	1.8	21.1
5	Continuous wheat	3.7	1.4	1.9	20.4

Wheat after two years of summerfallow. This sequence was included to determine whether two years of summerfallow would reduce the infection rating even more than one year of summerfallow. The results, which are given in Table XII, indicate that, under average conditions of this experiment, one year of summerfallow is as effective as two for the purpose indicated (treatments Nos. 12 and 1).

TABLE XII

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
12	Wheat after two years of summerfallow	1.9	1.7	2.4	29.8
1	Summerfallow alternating with wheat	1.9	1.7	2.2	27.1
5	Continuous wheat	3.7	1.4	1.9	20.4

One year of summerfallow followed by two crops of wheat. Although the average infection rating on the first crop of wheat after summerfallow was low, *viz.* 1.9, that on the second crop of wheat in this sequence was increased to 3.7 (treatments Nos. 1 and 13). Also the averages of the total number of culms and number of fertile culms, and yield were reduced, as indicated in Table XIII.

TABLE XIII

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
13	Second wheat crop after summerfallow	3.7	1.4	2.0	22.0
1	Summerfallow alternating with wheat	1.9	1.7	2.2	27.1
5	Continuous wheat	3.7	1.4	1.9	20.4

The data given show how quickly foot rot increases normally. From the standpoint of foot rots and also economy, a crop of oats the second year after summerfallow, where practicable, would provide a better sequence.

Summerfallow (manured) followed by two crops of wheat. Rosen and Elliott (45), and Fellows (16) state that stable manure, applied to soil infested with *O. graminis*, greatly reduced the severity of take-all. Peyronel (42) recommends nitrogenous fertilizers for this purpose. Moritz (36) did not observe consistent effects of such application, while Benoist and Bailly (5), Erhard-Frederiksen (14), and Tedin (62) state that manure favors the development of take-all. Apparently there are no very definite observations respecting the effect of manure on the foot rots caused by *H. sativum* or *Fusarium* spp.

In these experiments, the rating was practically as low the first year on manured summerfallow, as it was on the summerfallow without manure (treatments Nos. 11 and 10). The rating on the second crop of wheat on manured summerfallow was practically the same as for a similar sequence where no manure was applied (treatments Nos. 14 and 13). Possibly a more effective test would be to apply manure to a continuous wheat sequence. The comparison is shown in Table XIV.

TABLE XIV

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
11	First wheat crop after manured summerfallow	2.1	1.9	2.5	32.2
10	First wheat crop after summerfallow	1.9	1.8	2.4	27.4
14	Second wheat crop after manured summerfallow	3.8	1.5	2.0	24.8
13	Second wheat crop after summerfallow	3.7	1.4	2.0	22.0

From these data is is evident that the yield was slightly greater for the sequences in which manure was used than for those in which it was not used.

Summerfallow followed by wheat seeded down with western rye. The infection rating for wheat in this sequence (treatment No. 7), cannot be compared fairly with other sequences in this project. However, where wheat was sown after two years of western rye in treatment No. 8, the rating was 4.3, while it was 2.4 in treatment No. 7. In the latter instance the limited available soil moisture after the second year of western rye probably would affect severely the wheat and favor foot-rot under average conditions in the prairie provinces. On this account, and because of less foot rot, and a greater yield, perhaps a more suitable procedure would be to use sweet clover instead of western rye (treatment No. 16). The averages of infection rating, total number of culms and number of fertile culms, and yield for the treatments mentioned are given in Table XV.

TABLE XV

Treatment	Rotation	I.R.	F.C.	T.C.	Y.B.
7	Summerfallow followed by wheat with w. rye (six-year rotation)	2.4	1.7	2.2	30.5
8	Wheat after two years w. rye (six-year rotation)	4.3	1.3	2.0	25.1
17	Wheat after two years w. rye (three-year rotation)	3.9	1.4	2.1	22.2
16	Wheat after two years sweet clover (three-year rotation)	2.1	1.7	2.3	25.4

Cultural Practices

Parisot (39), and Hulsenberg (29) in Europe, state that take-all is favored where cultural practices provide conditions above the optimum for growth, while Tepper (63), and Pearson (40) of Australia, and Rosen and Elliott (45) at Arkansas, state that undernourished plants are more susceptible. Certain cultural practices, such as rate, date, depth of seeding, and firmness of soil, which might affect the vigor of the plants, were studied under field conditions.

The observations were made, in one instance on the second wheat crop after summerfallow (treatments Nos. 21–31, inclusive), and in the other case from wheat seeded on summerfallow (treatmeuts Nos. 32–42, inclusive). Since the infection rating was not significantly different for any of the sequences in which wheat followed summerfallow, except possibly for late seeding, discussion will be confined to the results at Indian Head, Swift Current and Scott (11 station-years) from seeding on spring-plowed wheat stubble. The check used for each cultural practice was the average for the station. There were 12 systematically distributed checks. The averages of infection rating, number of fertile culms and number of total culms, and yield were in each case very similar, as may be seen in the results from treatments Nos. 21, 26, and 31.

Date of seeding. Parisot (39), Brunchant (9), Gaudineau and Guyot (19), and Feistritzer (15), in Europe, and Kirby (32) in America, state that winter wheat, sown early, is more susceptible to take-all than if it is sown late. According to Russell (47, 48) this is also true of spring wheat. Apparently no very definite information regarding *H. sativum* and *Fusarium* spp. is available. The average results from three dates of planting (treatments Nos. 26, 27, and 28) are shown in Table XVI.

TABLE XVI

Treatment	Cultural practice	I.R.	F.C.	T.C.	Y.B.
26	Average	2.3	1.3	1.8	13.8
27	Late	1.7	1.7	1.7	13.9
28	Early	2.8	1.4	1.8	12.0

In spite of the reduced infection rating from planting late, this practice, on account of early frost hazard, would not be safe in western Canada, except possibly in southern districts.

Rate of seeding. Guerrapain and Demolon (24), Mangin (35), Foëx (17), Sévegrand (57), Parisot (39), Erhard-Frederiksen (14), and Peyronel (42) in Europe, and Kirby (32) in America, state that with winter wheat take-all is favored by dense sowing. Obviously, the effect of rate of seeding on

yield and vigor of the plants, may be modified greatly by available soil moisture and nutrients. The average results obtained from average, light, and heavy seedlings (treatments Nos. 21, 22 and 23) are compared in Table XVII.

TABLE XVII

Treat-ment	Cultural practice	I.R.	F.C.	T.C.	Y.B.
21	Average	2.4	1.3	1.7	13.5
22	Light	2.3	1.5	1.8	13.2
23	Heavy	2.4	1.2	1.5	13.8

Depth of seeding. Russell (47, 49) states that, in pot culture, plants from wheat grains planted 4 in. deep, suffered from take-all more than when they were planted 2 in. deep. With respect to foot rot caused by *H. sativum* or *Fusarium* spp., field observations frequently suggest that very deep seeding increases the disease. However, in these experiments no definite results were obtained, possibly because the differences between "shallow", "deep" and "average" were not sufficient for an adequate test. The average results (treatments Nos. 24, 25, and 26) are given in Table XVIII.

TABLE XVIII

Treat-ment	Cultural practice	I.R.	F.C.	T.C.	Y.B.
24	Deep	2.6	1.4	1.7	15.8
25	Shallow	2.4	1.4	1.7	13 4
26	Average	2.3	1.3	1.8	13.8

Packing. Sometimes farmers maintain that foot rot is reduced when a loose soil is packed. Russell (47), by controlling the soil moisture, found little effect on take-all from compacting the soil in pot culture, except that it retarded the emergence of the plants. The results in Table XIX obtained from heavy, light, and no packing of the soil, at the average rate, date, and depth of seeding (treatments Nos. 29, 30 and 31), were, under the conditions of the experiment, very similar.

TABLE XIX

Treat-ment	Cultural practice	I.R.	F.C.	T.C.	Y.B.
29	Heavy	2.8	1.4	1.8	14.4
30	Light	2.6	1.4	1.7	14.7
31	None	2.7	1.4	1.7	13.7

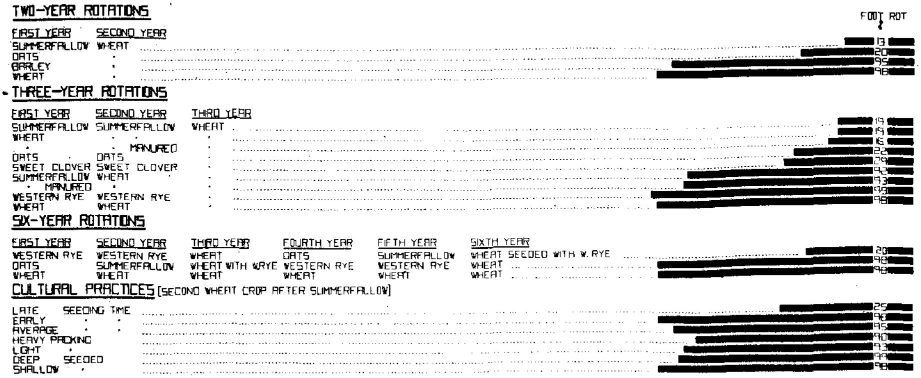

FIG. 2. *Effect of crop rotation and cultural practice on the development of foot rot of wheat at seven stations in western Canada for the years 1930 to 1932, inclusive.*

Graphical Summary of Infection Rating for Project

The summarized results, from the entire project for the years 1930 to 1932, inclusive, indicating the infection rating of wheat resulting from various crop sequences and cultural practices, are illustrated in Fig. 2. The percentages indicate the relative rating obtained and do not necessarily suggest the actual degree of loss.

Discussion

It may be well to recall the wide range of soil and climatic conditions under which the foregoing results were obtained, and also the associated advantages and difficulties. Foot-rotting fungi were not added to the soil, or their development unnaturally stimulated. Also, the crop rotations and cultural practices were carried out in a way quite comparable to ordinary farming methods. In spite of natural difficulties in a field project of this kind, and the fact that some of the crop sequences are still too young to permit of final conclusions, the results given are reasonably well founded, and have a wide application in the prairie provinces of Canada.

Foot rot always developed most where wheat followed wheat, barley, or western rye grass, which in general verifies field observations. Also, the average number of fertile culms and total culms, and yield were uniformly lower in these sequences than in any others used. There was a marked decrease in foot rot and an increase in the number of fertile culms, total number of culms, and yield where wheat followed summerfallow. Under the conditions of the experiment, two years of summerfallow did not appear to be more effective in reducing foot rot than one year, and hence this procedure would seem both unnecessary and unsound, except possibly in more severe cases of foot rot than those studied in this investigation. However, the average number of fertile culms, total number of culms, and the yield were increased significantly in each instance. Where a crop of oats preceded wheat, foot rot was reduced practically as much as it was by summerfallow, and, as with summerfallow, two crops of oats did not appear to be more effective than one crop in reducing the infection rating. The number of fertile culms, total number of culms, and yield were very similar in both cases, and not significantly different for the continuous wheat sequence. Where wheat followed two crops of sweet clover the general effect on foot rot was the same as that obtained by summerfallow or oats. However, the yield, number of fertile culms, and total number of culms were increased over those observed where wheat followed oats.

The application of manure to summerfallow did not, in these studies, significantly modify the amount of foot rot on the two subsequent crops of wheat, beyond what occurred on similar sequences without manure. However, it is felt that these results should not be accepted as final for all conditions. The possibility that manure, applied to a continuous wheat sequence, would favor foot rot more than it does when applied to summerfallow, should not be overlooked.

Concerning the cultural practices available to the farmer, such as rate, date, and depth of seeding, and packing of the soil, the only evidence from these studies was that foot rot develops less on plants from late-sown wheat than on those from earlier seedings. However, this method cannot be safely used in western Canada because of frost hazard.

Finally, it should be mentioned that in these studies the progress of the foot rot was based wholly on the condition of the crown and secondary root tissue, and that the condition of the spike or the grains was not considered. The experimental evidence given earlier suggested this procedure as being the more reliable. Also, field observations of the foot rots, each year, indicate that frequently the progress of this disease in the crown may be marked, and yet the spike and grains may or may not reflect a corresponding effect.

Acknowledgments

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

IV. EFFECT OF CROP ROTATION AND CULTURAL PRACTICE ON THE RELATIVE PREVALENCE OF *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM* SPP. AS INDICATED BY ISOLATIONS FROM WHEAT PLANTS[1]

By W. C. Broadfoot[2]

Abstract

The crown and root tissue from 43,305 of 47,360 plants examined in this investigation yielded *Helminthosporium sativum*, *Fusarium culmorum* and other *Fusarium* spp., either alone or in combination with these or other fungi and bacteria. It was the exception for any mature plant, the surface tissue of which was disinfected, to be free from fungi or bacteria. None of the various crop sequences or cultural practices used in this study appeared to significantly affect more than another the relative prevalence of either *H. sativum* or *Fusarium* spp., as indicated by isolations from the crown tissue of wheat. However, as there was a marked tendency at certain stations each year for *H. sativum* or *Fusarium* spp. to predominate, it was concluded that certain factors of the environment were more effective than the crop sequence in modifying the relative prevalence of the two fungi mentioned in the crown and root tissue of wheat plants.

Introduction

Commonly either *Helminthosporium sativum* P.K. and B. or *Fusarium* spp., or both, grow from the crowns and other basal parts of wheat plants suffering from foot rot, and yet they may or may not be parasitic. Ordinarily, these fungi, when the tissue is surface disinfected and incubated on solid media, grow to the exclusion of *Ophiobolus graminis* Sacc., even when the latter has caused most of the damage. On the other hand, *H. sativum* and *Fusarium* spp. are important foot-rotting pathogens, and they are extremely common in the soils of western Canada. However, there appear to be no very definite data in the literature as to whether the relative proportion of these are modified by certain crop rotations, cultural practices, regional location, climatic or other factors. Opportunity to obtain information on these interesting possibilities was afforded by the material available in the crop sequence and cultural studies which are described in Part III of this series (1).

Material and Methods

A preliminary experiment was made to determine whether isolating from the crown tissue would indicate reasonably well the prevailing fungus in both crown and secondary roots. For this purpose an average of 46 plants was selected at random in 1928 on the long-time, continuous wheat plots at each of the following stations:—Brandon, Indian Head, Swift Current, Lethbridge,

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and Lacombe. Sections were cut from the roots more than 1 cm. from the crown, and from 1 cm. next to the crown. The crown was also cut longitudinally in halves. After disinfecting for two minutes in a 1 : 1000 mercuric chloride solution, these portions were placed on potato sucrose agar in separate plates. Half of each crown was placed cut side down, and the other half cut side up. After 10 days at room temperature, the colonies of *H. sativum*, *F. culmorum*, and other species of *Fusarium* growing from the sections were recorded for each plant part. In general, the identification of the fungi was based on macroscopic characters.

In 201 out of 280 cases, or 87.4%, apparently the same organism grew from both halves of the crown. In 186 out of 230 cases, or 80.9%, the same organism grew from the roots 1 cm. next to the crown as grew from the crown tissue. In 155 out of 230 cases, or 67.4%, apparently the same fungus inhabited the roots more than 1 cm. from the crown as was isolated from the crown. Of particular interest is the fairly close agreement in kind of organism in the two halves of the crown and in the roots 1 cm. long next to the crown. The correlation seemed to decrease in sections of the root more remote from the crown. Hence, in spite of certain variations it seemed best and reasonably safe, for the purposes of this study, to culture from the crown tissue only. The routine procedure consisted in taking the main tiller, removing the secondary roots close to the crown, the subcrown internode about 1 cm. below it, and also the culm about 1 cm. above the crown. During 1928 and 1929, determinations were made on only 10 random plants from each replicate of a sequence or practice, but from 20 plants in 1930, 1931 and 1932.

The crown parts were disinfected in mercuric chloride solution as before for two minutes, and incubated for 12 days in Petri plates on potato sucrose agar. The colonies of *H. sativum*, *F. culmorum* and other *Fusarium* spp. were recorded, and some other fungi were identified.

Owing to the practical impossibility of identifying with certainty all isolations of what apparently was *H. sativum* and *F. culmorum*, not to mention other *Fusarium* spp., in a study of this magnitude, the following comparisons will be based only on the isolations tentatively identified as *H. sativum* and *Fusarium* of the *culmorum* type and other *Fusarium* spp. While there may have been cases of mistaken identity of both fungi mentioned, the classification made of the two fungi, so different in color and general growth characteristics, will probably be sufficiently accurate for the purposes of this study.

Because of difficulties mentioned, there is doubt about the wisdom of attempting to classify even roughly the *culmorum* type of *Fusarium* from among the *Fusarium* species present. However, the results obtained indicate how common this type is in the crown tissue of wheat, while not obscuring the relative prevalence of *H. sativum*, or the *Fusarium* spp. as a group.

The results, based on the total colonies of both *H. sativum* and *Fusarium* spp. will be given to the nearest decimal in percentage for *H. sativum* and *F. culmorum*. The percentage of other or all *Fusarium* spp. can be estimated readily where not listed.

Experimental Results

Fungi Commonly Isolated

The crown and root tissue from 43,305 of 47,360 plants examined in this study yielded *H. sativum*, *F. culmorum* and *Fusarium* spp., either alone or in combination with these and other identified and unidentified fungi and bacteria, so that it may be safely said that it was the exception for any mature plant, the surface tissue of which was treated, to be free from fungi or bacteria. *H. sativum* and *Fusarium* of the *culmorum* type, and also other *Fusarium* spp. were usually isolated from plants, the basal tissue of which bore signs of foot-rot infection. Sometimes one or more of the following grew from the tissue:—*H. sativum*, *Fusarium* spp., *Penicillia*, *Alternaria*, *Rhizoctonia* sp., *Macrosporium* sp., *Cladosporia*, *Actinomyces* sp., *Hormodendrum* sp., *Wojnowicia graminis*, *Ascochyta graminicola*. *Aspergillus niger* and *Monilia sitophilia* frequently grew from the plated tissue, and often to the exclusion of other fungi or bacteria. However, in most cases, the tissue yielded either *H. sativum* or *Fusarium* spp. or both.

In spite of the fact that signs of take-all were evident on occasional wheat plants in all experiments, and at certain stations were more or less pronounced, *O. graminis* was not definitely identified once from 47,000 odd plants plated out. Its absence may be accounted for partly by the presence of other micro-organisms that grow more rapidly in plate culture, by the rather unsuitable substrate that was used, by the slow growth-habit of this fungus, or by the disinfectant used.

From observations, during this study, the opportunities for *Fusarium* spp. and *H. sativum* to grow from the plated tissue would seem equally good. These fungi grow vigorously and are not very definitely antagonistic to each other, and any advantage in competition on the substrate used is usually with the *Fusarium* spp.

Variation in Results

It may be well to supply here, from the data, a typical example of the variation in percentage, of *H. sativum* and *Fusarium* spp., which occurred between the means of two similar crop sequences at a station in a given year. The results from material from the two continuous wheat sequences (treatments Nos. 5 and 6) at seven stations in 1930, will be used for the purpose. The average relative prevalence in percentage of *H. sativum*, *F. culmorum* and other species of *Fusarium* is indicated in Table I.

In both sequences, the variation with respect to *H. sativum* exceeded 10% at four of the seven stations, that is to say, this fungus appeared to be less prevalent in one of two identical rotations than the other at one station. A good example of this was at Olds, where the percentage was approximately 2 with treatment No. 6, and 19 with treatment No. 5. The cause for this variation is not known. However, there was fairly good agreement when the data from all stations were averaged. A similar situation existed with regard to *F. culmorum*, and to other species of this genus. At one of the stations the

TABLE I

PERCENTAGE ISOLATIONS OF *Helminthosporium sativum*, *Fusarium (culmorum* type) AND ALL *Fusarium* spp., FROM WHEAT OF TWO IDENTICAL SEQUENCES OF CONTINUOUS WHEAT AT VARIOUS STATIONS IN 1930

Treatment	Stations*							
	Mord.	I.H.	S.C.	Scott	Leth.	Olds	Verm.	Av.
(H. sativum)								
5. Cont. wheat	37	62	46	63	53	19	49	47
6. Cont. wheat	48	76	37	60	52	2	66	48
(F. culmorum)								
5. Cont. wheat	17	16	27	2	26	65	4	23
6. Cont. wheat	9	10	31	12	30	79	2	25
(All Fusarium spp.)								
5. Cont. wheat	63	38	54	37	47	81	51	53
6. Cont. wheat	52	24	63	40	48	98	34	52

**Following are abbreviations in above and in subsequent tables: Morden (Mord.), Manitoba; Indian Head (I.H.), Swift Current (S.C.), and Scott in Saskatchewan; and Lethbridge (Leth.), Olds and Vermilion (Verm.) in Alberta.*

variation was less, and in some cases probably within the error of the experiment. In the case of all *Fusarium* spp. the variation was greater than 11% at four of the seven stations. The most significant thing about this is that in the same year either *F. culmorum* or all species of *Fusarium* might be relatively scarce at one station and relatively abundant at another station in a similar sequence. This is well illustrated by the results from Vermilion and Olds.

Variation at all Stations each Year

The two similar crop sequences, which were used to illustrate the variation in results occurring among stations in a given year, will be employed to indicate the yearly variation in the average results from all stations. The average relative prevalence of *H. sativum*, *F. culmorum* and other Fusaria is indicated in percentage in Table II.

TABLE II

AVERAGE YEARLY PERCENTAGE ISOLATIONS FROM ALL STATIONS OF *Helminthosporium sativum*, *Fusarium (culmorum* type) AND OTHER *Fusarium* spp. FROM WHEAT, OF TWO IDENTICAL SEQUENCES OF CONTINUOUS WHEAT

	Sequence No. 5					Sequence No. 6				
	1928	1929	1930	1931	1932	1928	1929	1930	1931	1932
H. sativum	51	47	47	35	52	48	46	49	29	51
F. culmorum	8	28	23	24	24	11	31	25	29	24
Other *Fusarium* spp.	41	25	30	41	24	41	23	26	42	25

Except in 1931, the average prevalence of *H. sativum* and *Fusarium* spp. was relatively constant, varying fairly closely around 50% in each crop sequence. In 1931 the number of *Fusarium* spp. was greater than *H. sativum*

by about 30% in sequence No. 5 and by 42% in sequence No. 6. The average of the results during five years of each of the two sequences would suggest similar values for these fungi.

Comparisons of other crop sequences, where various crops precede wheat, cannot be made for obvious reasons. Even considerable variations might be expected in such instances, although it will be apparent later that the example just given fairly well illustrates the kind and range of variation in the data throughout the study.

The Relative Prevalence of *H. sativum* and *Fusarium spp.* as Isolated from Wheat in Various Crop Sequences and Cultural Practices

Christensen (2) and others have shown the constant occurrence of *H. sativum* on the basal parts of barley, and Simmonds (3) the occurrence of *F. culmorum* on the crown and root tissue of oats. The crop sequences from which wheat plants were obtained for isolation purposes in this study are as follows, each with its respective reference number:—*Two-year rotations:* 1, summerfallow, wheat; 2, oats, wheat; 3, barley, wheat; 5, wheat, wheat; 6, wheat, wheat. *Three-year rotations:* 10, wheat, summerfallow, wheat; 11, wheat, summerfallow (manured), wheat; 12, summerfallow, summerfallow, wheat; 13, summerfallow, wheat, wheat; 14, summerfallow (manured), wheat, wheat; 15, oats, oats, wheat; 16, sweet clover, sweet clover, wheat; 17, western rye, western rye, wheat.

The foregoing rotations were at five of the Dominion Experimental Stations, and at two of the Alberta Schools of Agriculture.

TABLE III

THE AVERAGE PERCENTAGE ISOLATIONS*, AT VARIOUS STATIONS†, OF *Helminthosporium sativum* AND OF *Fusarium culmorum* FROM WHEAT WHICH FOLLOWED DIFFERENT CROPS

Station	Mord.		I.H.		S.C.		Scott		Leth.		Olds		Verm.		Av.	
Treatment	Hs	Fc	Hs	Fc	Hs	Fc	Hs	Fc	Hs	Fc	Hs	Fc	Hs	Fc	Hs	Fc
Wheat after:																
1. S. fallow	56	13	44	32	37	26	41	22	44	31	39	34	47	8	44	24
2. Oats	57	14	48	30	37	27	29	29	45	35	18	51	46	9	40	28
3. Barley	60	8	58	23	48	22	40	20	47	31	28	37	51	10	47	22
5. Wheat	55	15	47	23	39	25	46	12	46	35	34	36	52	5	46	21
6. Wheat	55	17	56	22	34	24	42	14	42	39	31	42	53	13	45	24
15. Two crops oats	52	23	52	23	36	30	31	44	36	35	20	74	38	19	38	36
16. Two years s. clover	51	17	46	27	36	19	40	27	36	36	8	79	36	4	36	30
17. Two years w. rye	50	27	60	17	50	14	43	19	46	35	11	60	44	12	44	26
Average	54	17	51	25	39	23	39	23	43	35	24	51	46	10	43	26

**H. sativum and F. culmorum indicated by "Hs" and Fc," respectively.*
†*For Mord., I. H., Scott and Leth., 1928 to 1932, inclusive; for S.C., 1928 to 1930, inclusive; and for Olds and Verm., 1930 to 1932, inclusive.*

The average results in Table III would suggest at first that at some of the stations *H. sativum* was isolated more frequently from wheat after certain crops than after others. For example, at Morden the percentage was 60 after barley and 52 after two years of oats, and 56 following summerfallow, and only 55% following continuous wheat. However, at other stations it is

obvious that this relation is not maintained. *H. sativum* appeared to be more frequently isolated from wheat after barley than from wheat after other crops, this being most marked at Indian Head and Swift Current. However, as its relative prevalence was almost as great, or sometimes much greater, on wheat after summerfallow, or after oats, as it was on wheat after wheat or after barley, the differences would seem within the experimental error. The variation indicating the relative prevalence of all *Fusarium* spp. as affected by the crop sequence, may be explained similarly. Although the figures for this group are not given in Table III, they can be calculated readily. There appears to be a definite tendency at some stations for either *H. sativum* or the *Fusarium* spp. to predominate, regardless of crop sequence. At Morden, the former was in the ascendancy, while at Indian Head and Vermilion they were about equally prevalent.

Average data in Table III also suggest that the *culmorum* type of *Fusaria* was isolated more frequently at certain stations than at others. For example, at Vermilion and Morden it was consistently lower than at any other station, irrespective of the crop sequence. At other stations the proportion of *F. culmorum* to all *Fusarium* spp. appeared to be relatively higher. It is repeated here that, on account of the difficulty of correctly identifying *F. culmorum*, only marked differences would be significant.

That the variation obtained may be due to conditions at a station, and not to any particular crop sequence, is further suggested by the data in Table IV, where the average results for various sequences are given for each year at all stations. In general, the proportion of *H. sativum* to *Fusarium* spp. is very uniform for all crops, with one possible exception, *viz.*, wheat after oats, where the trend is for a greater proportion of *Fusarium* spp. However, greatest differences, which appear to be more consistent, occurred in certain years, for example, in 1931, where the isolations of *Fusarium* spp.

TABLE IV

THE YEARLY AVERAGE PERCENTAGE (FOR ALL STATIONS) OF ISOLATIONS* OF *Helminthosporium sativum* AND OF *Fusarium* spp., FROM WHEAT IN VARIOUS CROP SEQUENCES†

Treatment	1928		1929		1930		1931		1932		Av.	
	H	F	H	F	H	F	H	F	H	F	H	F
Wheat after:												
1. Summerfallow	50	50	41	59	49	51	34	66	49	51	45	55
2. Oats	54	46	40	60	43	57	31	69	37	63	41	59
3. Barley	56	44	50	50	48	52	37	63	49	51	48	52
5. Wheat	51	49	47	53	47	53	35	65	52	48	46	54
6. Wheat	48	52	46	54	49	51	29	71	51	49	45	55
15. Oats, oats	43	57	41	59	41	59	27	73	41	59	39	61
16. S. clover, s. clover	38	62	45	55	42	58	30	70	39	61	39	61
17. W. rye, w. rye	52	48	54	46	48	52	33	67	49	51	47	53
Average	49	51	45	55	46	54	32	68	46	54	44	56

**H. sativum and Fusarium spp. indicated by "H" and "F," respectively.*
†*Treatments Nos. 1, 2 and 3 are two-year rotations; Nos. 15, 16 and 17 three-year rotations; and Nos. 5 and 6, continuous wheat.*

predominated over those of *H. sativum* to a greater extent than for any year of the study. Similarly, in 1928, *H. sativum* was predominant, while in other years it was more or less secondary.

Effect of Manure

In this study, isolations of *H. sativum* and *F. culmorum* were made from wheat in the four following three-year rotations:—10, wheat, summerfallow, wheat; 11, wheat, summerfallow (manured, 12 tons), wheat; 13, summerfallow, wheat, wheat; and 14, summerfallow (manured, 12 tons), wheat, wheat. The only difference between the first two rotations and between the last two rotations was that manure was applied to the summerfallow in one sequence in each instance. The infection rating on the third and fourth sequences, *viz.*, 13 and 14, where the isolations were made from the second crop of wheat, was 3.5 and 3.5, respectively, while for the first two, *viz.*, 10 and 11, it was 1.9 and 2.2.

TABLE V

THE EFFECT OF MANURE ON THE AVERAGE PERCENTAGE ISOLATIONS* OF *Helminthosporium sativum* AND OF *Fusarium* spp. FROM WHEAT. THE DATA APPLY TO CROP SEQUENCES AT MORDEN, INDIAN HEAD AND SCOTT, 1928 TO 1932, INCLUSIVE

Stations	Mord.		I.H.		Scott		Mean av.	
Treatment	H	F	H	F	H	F	H	F
Wheat after:								
10. Summerfallow	46	54	53	47	45	55	48	52
11. Summerfallow (manured)	50	50	55	45	36	64	47	53
13. Summerfallow, wheat	52	48	62	38	52	48	56	44
14. Summerfallow (manured), wheat	49	51	55	45	51	49	51	49

**H. sativum and Fusarium spp. indicated by "H" and "F," respectively.*

In Table V are shown the average percentages of *H. sativum* and *Fusarium* spp., obtained at three stations from 1928 to 1932, inclusive. It may be stated briefly that the results in this table indicate that the proportion of *H. sativum* to all *Fusarium* spp. was about the same where manure was applied as where it was not applied. The slight difference in either direction is probably not significant.

Effect of Cultural Practices

Additional evidence that *H. sativum* and *Fusarium* spp. may be isolated from wheat by the method used, in about the same relative proportions, irrespective of crop sequence or cultural practice, is supplied by summarized data from that part of the experiment described in Part III (1) under "Cultural Practices", (treatments Nos. 21 to 42, inclusive). In this experiment the date, depth and rate of seeding wheat were each varied three ways, that

is to say, the seed was sown early, average and late; deep, average and shallow; and at a light, average and heavy rate. This experiment was duplicated on two rotations, namely "wheat, summerfallow, wheat"; and "summerfallow, wheat, wheat". The isolations in the latter instance were made from the second crop of wheat. The proportion of *H. sativum* to *Fusarium* spp., isolated using the various practices mentioned, is given in Table VI. Here it is apparent that the numbers of *H. sativum* and the *Fusarium* spp. isolated bear the same general ratio in each of the sequences at each of the three stations.

TABLE VI

THE AVERAGE PREVALENCE IN PERCENTAGE OF *Helminthosporium sativum** and *Fusarium* spp.* AS ISOLATED *A*, FROM THE SECOND WHEAT CROP AFTER SUMMERFALLOW, AND *B*, FROM THE FIRST WHEAT CROP AFTER SUMMERFALLOW, WHERE THE DATE, RATE, AND DEPTH OF SEEDING WERE VARIED

Station	*A*								*B*							
	I.H.†		S.C.‡		Scott†		Av.		I.H.†		S.C.‡		Scott†		Av.	
	H	F	H	F	H	F	H	F	H	F	H	F	H	F	H	F
Date of seeding																
Average	51	49	50	50	42	58	48	52	54	46	39	61	46	54	46	54
Late	60	40	40	60	47	53	49	51	57	43	45	55	54	46	52	48
Early	54	46	42	58	54	46	50	50	51	49	51	49	51	49	51	49
Rate of seeding																
Average	46	54	44	56	46	54	45	55	52	48	38	62	53	47	48	52
Light	53	47	41	59	49	51	48	52	55	45	50	50	58	42	55	45
Heavy	58	42	49	51	50	50	52	48	54	46	50	50	53	47	52	48
Depth of seeding																
Average	60	40	40	60	47	53	49	51	57	43	45	55	54	46	52	48
Deep	55	45	51	49	46	54	51	49	57	43	39	61	56	44	51	49
Shallow	51	49	50	50	43	57	48	52	54	46	39	61	46	54	46	54
Average	54	46	45	55	47	53	49	51	55	45	44	56	52	48	50	50

**Indicated by "H" and "F," respectively.* †*A four-year average (1928-1931).*
‡*A three-year average (1928-1930).*

Effect of Climatic and Soil Factors

So far in this study, the crop sequence or cultural practice did not seem to modify significantly the proportion of *H. sativum* to *Fusarium* spp. That soil or climatic factors may have some effect is suggested in Table VII where the yearly average preponderance for the respective fungi is indicated in percentage.

TABLE VII

THE EXCESS, IN PERCENTAGE, OF ISOLATIONS* OF *Helminthosporium sativum* AND *Fusarium* spp., OR *vice versa*, AT EACH STATION† DURING 1928 TO 1932, INCLUSIVE. DATA ARE BASED ON AVERAGE ISOLATIONS FROM WHEAT IN ALL SEQUENCES

Station	1928		1929		1930		1931		1932		Average	
	H	F	H	F	H	F	H	F	H	F	H	F
Mord.	6		14		14			34	2		0	0
I.H.	4			2	34			22	—	—	4	
S.C.	0	0		10		26	—	—	—	—		12
Scott		2		12	14			26		4		6
Leth.	16			14		6		32		44		16
Olds	—	—	—	—		74		44	6			37
Verm.	—	—	—	—	4			24		10		10
Av.	5			5		6		30		10		9

**H. sativum* and *Fusarium* spp. indicated by "H" and "F," respectively.
†Data are absent where dashes occur.

In 1928 the isolations of *H. sativum* were greater than those of *Fusarium* spp. at Lethbridge, Indian Head, and Morden (16, 4 and 6%, respectively), but at Scott the difference was not significant. In 1929 the isolations were from 2 to 14% greater at all stations, except Morden, where those of *H. sativum* were greater (14%). In 1930, when final data from Olds and Vermilion were available, making seven stations in all, *H. sativum* was apparently more abundant at Indian Head, Scott, and Morden by 34, 14, and 14%, respectively, and at Vermilion by 4%, while the *Fusarium* spp. were in the majority at Olds by 74%, at Swift Current by 26%, and at Lethbridge by only 6%. The most striking difference was in 1931, when the isolations of *Fusarium* varied from 22 to 44% greater at the seven stations. In 1932 each organism appeared to be about equally abundant at all stations, except at Lethbridge, where the advantage for the *Fusarium* species was 44%. Data for Indian Head and Swift Current were not available for 1932.

Although it is impossible here to offer the correct explanation for the yearly variation indicated above, it would seem that the difference in soil and climate among the various stations might, either directly or indirectly, be responsible.

Discussion

In view of the nature of the problem, and the necessarily indirect methods of analysis employed, only trends in certain directions rather than sharply defined results were anticipated. The fact that the tissue from which the platings were made was located in the soil, and therefore subject, not only to a range of soil conditions, but invasion by saprophytic as well as parasitic fungi, introduces conflicting factors. Then too, in artificial culture, the characteristic growth habits in association, of the fungi which may emerge from the internal tissue, not affected by the disinfectant, may tend to render difficult, and at times perhaps make impossible, the recognition of certain fungi. However it was pointed out earlier that, in general, both *H. sativum*

and the *Fusarium* spp. are about equally vigorous in growth, and fairly compatible. Moreover the regularity with which one or the other, and often both of these fungi emerged from the crown tissue used, suggests that a fairly accurate picture might be obtained. Obviously, without tests for pathogenicity, we have no idea regarding the possible significance of their presence, nor can the relative prevalence of these fungi in the tissue more than suggest their relative prevalence in the soil.

In this study the crown portions of 47,360 plants were cultured on potato sucrose agar. Either *H. sativum* or *Fusarium* spp., or both were found on 43,305 plants, which is very significant in view of the possible injury from toxic growth products and general parasitic effects in this tissue.

As far as could be ascertained from this study, the crop sequence or cultural practice used did not appear to affect significantly the relative prevalence of either *H. sativum* or *Fusarium* spp. in the crown tissue of wheat at a given station in any year. This is interesting in view of the fact that summerfallow, a crop of oats, sweet clover, or some non-susceptible crop, actually reduced the foot-rot damage on the following crop of spring wheats.

Perhaps the most significant result obtained was the indication that the climatic conditions, and possibly the soil, at a station, each year, determined the relative prevalence of *H. sativum* and the *Fusarium* spp.

Acknowledgments

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CYTOSPORA INFECTION FOLLOWING FIRE INJURY IN WESTERN BRITISH COLUMBIA[1]

BY JOHN DEARNESS[2] AND J. R. HANSBROUGH[3]

Abstract

Data are given concerning the appearance of *Cytospora* spp. on 15 species of shrubs and trees following a light ground fire near D'Arcy, B.C. On 11 of the hosts was found a species of *Cytospora*, which is herein described under the name, *C. pulcherrima*. On three of the hosts was found an undetermined *Cytospora* with larger spores and darker, less delicate tendrils. On the one coniferous host present, Douglas fir, the only fungus present was determined as *C. friesii* Sacc.

The taxonomic importance of the study rests on the range of species of infected hosts which revealed a width hitherto unknown or at least unreported for a *Cytospora*.

In the north temperate latitudes there are few, if any, lignicolous plant species which are exempt from invasion by one or more parasitic or saprophytic members of the genus *Cytospora*. Thus, *Cytospora* spp. are common and sometimes highly injurious parasites of poplar (*Populus* spp.) (2, 5, 6, 7, 9, 10–14) and there are several published reports (4, 8, 15) of serious canker injury to fruit trees by the same or closely related species. The majority, however, of the species in this genus are regarded as saprophytes, or at worst, as parasites upon weakened living tissue. An example of this type of injury was observed by the junior writer and J. L. Mielke at D'Arcy, B.C.,* on May 31, 1931. The circumstances surrounding the case were thought to be of sufficient interest to merit a brief study of the conditions antecedent to the infection and of the fungi involved.

In the early spring of 1931 a light ground fire had run over an area of approximately one-tenth of an acre and had killed most of the herbaceous annuals and injured all of the shrubs and small trees. Of this latter group there were 15 different species present, and on all of them *Cytospora* spp. were fruiting near the bases of the living but weakened stems. The hosts, listed in the order of their apparent susceptibility to infection, were as follows: *Salix* sp., *Alnus tenuifolia* Nutt., *Populus trichocarpa hastata* Henry, *Philadelphus gordonianus* Lindl., *Cornus occidentalis* (T. and G.) Cov., *Crataegus brevispina* (Doug.) Hell., *Betula fontinalis* Sarg., *Pseudotsuga taxifolia* (LaM.) Britt., *Acer glabrum douglasii* (Hook.) Dipp., *Amelanchier florida* Lindl., *Grossularia divaricata* (Doug.) Cov. and Britt., *Ribes sanguineum* Pursh, *Rosa nutkana* Presl., *Sambucus glauca* Nutt., and *Rubus leucodermis* Doug.

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**D'Arcy is a stop on the Pacific Great Eastern Railway approximately 90 miles north of Vancouver, B.C.*

This susceptibility rating is based on the estimated bark area infected on the various species. No definite information could be obtained as to whether or not all of the host species were uniformly injured by the fire but from general observation such was thought to be the case. All of the shrubs and saplings on the area were rather badly scorched but in no instance had death resulted. Therefore, for example, if the willow and maple were apparently equally injured by the fire but the former was much more heavily infected by the fungi, then it was considered to be more susceptible to such invasion.

The typical cirri, or spore tendrils, of the genus *Cytospora* were very noticeable on all of the scorched plants except the Douglas fir, and were particularly conspicuous on the willow and alder saplings where they hung in moss-like appendages, three to four inches in length.

Collections of the fungi on all 15 hosts were made and sent to the senior author for critical study and determination.

Allescher (1) gives descriptions of 171 species of *Cytospora* reported from Central Europe, many of whose descriptions are practically duplicated, or even triplicated, in their important features except host habitat. It is reasonable to expect that many of these so-called species will, when their life histories are fully known, be reduced to synonomy or varietal rank. The variation in the thickness, firmness and chemical nature of the bark of the different hosts may be expected to cause some corresponding variation in the morphology of a single fungus growing upon them.

If no two of the 15 hosts enumerated above had grown within miles of each other it is probable that instead of one species of *Cytospora* appearing on 11 of them, there might have been at least a few supposedly different species. The identical conditions under which these examples were initiated and developed give them unusual taxonomic importance. The question arises whether or not the scorching of the bark is a favoring or possibly a necessary condition of its growth.

As indicated above, on 11 of the 15 host species there was found the same *Cytospora* which is described below as a new species.

Cytospora pulcherrima

Cytospora pulcherrima sp. nov., but possibly a stage of *Valsella pulcherrima* (E. and E.) Berl. (3).

Pustules conic, rather thinly and evenly scattered, less frequently in small groups, concolorous but gradually darkening as the underlying stromata progress toward maturity, showing a whitish granular substance around the perforation at the apex. Stromata erumpent, seated in the cortex, not reaching the wood, not circumscribed by a dark line; flesh pallid at first, becoming dark gray, 1–1.3 mm. in diameter at base; pycnidial chambers few to many, radiate-crowded, opening into a common, black, minute ostiole which when mature enough to discharge conidia can be seen with a hand lens as a dark speck in the white granular apex. Conidia allantoid, hyaline, minute, 3.5–4

by 0.75 μ, issuing in protected places as pale amber-colored, hair-like tendrils, elsewhere as darker corkscrews, horns or beads. Conidiophores filiform, fasciculate, or subdendriform; forming a layer over the hymenial lining of the chambers, 15–22 μ deep.

On Salicaceae; *Salix* sp., *Populus trichocarpa hastata:* Betulaceae; *Betula fontinalis, Alnus tenuifolia:* Cornaceae; *Cornus occidentalis:* Rosaceae: *Amelanchier florida, Crataegus brevispina, Rosa nutkana:* Saxifragaceae; *Philadelphus gordonianus:* Aceraceae; *Acer glabrum douglasii:* Caprifoliaceae; *Sambucus glauca.*

Type locality. D'Arcy, British Columbia.

Distribution. Known only from the type locality and from near Northport, Washington.

Specimens examined. Herbarium of the Division of Forest Pathology, United States Department of Agriculture: Collections as follows made at D'Arcy, B.C., by J. R. Hansbrough and J. L. Mielke. On *Salix* sp. 40,844. On *Alnus tenuifolia*, 40,845. On *Populus trichocarpa hastata*, 40,846. On *Cornus occidentalis*, 40,847. On *Crataegus brevispina*, 40,848. On *Philadelphus gordonianus*, 40,849. On *Betula fontinalis*, 40,850. On *Acer glabrum douglasii*, 40,852. On *Amelanchier florida*, 40,853. On *Rosa nutkana*, 40,856. On *Sambucus glauca*, 40,857.

Herbarium of J. Dearness: Parts of all the above collections under one group number, 7,859 (Composite type). On *Salix* sp., Pepoon Lake, Northport, Wash., coll. E. E. Hubert.

On all of the hosts at D'Arcy except the *Rosa* and *Sambucus*, the *Cytospora* was closely associated with a Valsella stage. Mature discharged ascospores of this *Valsella* were not observed but their size and shape in the ascus were determined and the fungus was identified as *V. pulcherrima*.

The *Valsella* at first closely tesselates the bark with black-margined disks, each centered with a speck of whitish granular substance. The regularity and prettiness of these disks at this stage probably suggested the specific name *pulcherrima*. Later they become conical pustules, with few to many crowded ostioles, not rising above the ruptured bark. In most of the pustules there is more or less whitish ectostromatic tissue overlying the perithecia and surrounding the ostioles.

In 1929 Prof. E. E. Hubert of the School of Forestry, Moscow, Idaho, sent the senior author a *Valsella*, which the latter referred to *V. pulcherrima*, and also a *Cytospora*, both collected on *Salix* sp. on the shores of Pepoon Lake, near Northport, Wash. Prof. Hubert reported the fungi as killing many clumps of the willow. These two forms cannot now be separated from the specimens from D'Arcy, B.C. The tentatively assumed relationship between the *Cytospora* and the *Valsella* remains to be proved but the assumption that they are related is so strong that the authors risk giving this important "Imperfect" the possibly provisional name of *C. pulcherrima*.

On three of the remaining four hosts, namely, *Grossularia divaricata*, *Ribes sanguineum* and *Rubus leucodermis*, there was present a second and entirely different *Cytospora* which has not been identified because of the absence of any perfect stage associated with it. This fungus has larger spores, 6–8 $\times$ 2 μ, and darker, redder, less delicate cirri. In addition to the three above-listed hosts, it was occasionally found along with *C. pulcherrima* on the 11 hosts upon which that fungus was collected.

On the one remaining host, *Pseudotsuga taxifolia*, the only fungus present was identified as *Cytospora friesii* Sacc. Considering the ubiquitous occurrence of some species of *Cytospora* it is significant that there was in this case no crossing-over of a species from a deciduous to a coniferous host, or *vice versa*.

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MEASUREMENTS OF THE SURFACE TENSION OF CARBON TETRACHLORIDE AT LOW TEMPERATURES[1]

By T. Alty[2] and G. F. Clark[3]

Abstract

The variation of the surface tension of carbon tetrachloride with temperature has been determined with considerable accuracy between +12° C. and −10° C.

Introduction

At temperatures higher than room temperature the surface tension of carbon tetrachloride has been determined by a number of observers, but at lower temperatures only a few isolated determinations (3, 4, 5) have been made. Accurate values of the surface tension over a range of temperature in the neighborhood of 0° C. were required in connection with other work in progress in this laboratory, and as these were not otherwise available, measurements of the surface tension have been made in the temperature range between +12° C. and −10° C. The present paper gives the results obtained.

Experimental

The two most accurate methods for the determination of surface tension are the capillary rise and the drop weight methods. The latter was found more convenient for the present work and was used throughout.

Harkins and Brown (2) have shown that the mass (m grams) of the drop which falls from a circular tip of radius R cm. is given by the formula

$$mg = 2\pi R\gamma f\left(\frac{R}{V^{\frac{1}{3}}}\right) \qquad (1)$$

where γ is the surface tension of the liquid in dynes per cm., V is the volume of the drop in cc. and $f\left(\frac{R}{V^{\frac{1}{3}}}\right)$ is a correction factor whose values are tabulated for different values of $\left(\frac{R}{V^{\frac{1}{3}}}\right)$ by Harkins and Brown.

In order that Equation (1) shall apply accurately, the tip on which the drop is formed must be perfectly circular and free from flaws and its lower surface, from which the drop hangs, must be perfectly horizontal. In addition, the rate of formation of the drop must be sufficiently slow. If due attention is given to these points, very accurate values of the surface tension may be obtained by this method.

The arrangement of apparatus used was quite similar to that of Harkins and Brown. As they have given a detailed description of it in their paper (1),

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this need not be repeated here. The drops fall into a weighing bottle of known mass which is supported inside a water-tight container. The latter is immersed in a large thermostat so that the weighing bottle acquires the temperature of the thermostat but is not wetted by the thermostat liquid. When temperature equilibrium is established a number of drops of tetrachloride are allowed to form slowly on the tip and are collected in the weighing bottle which is then removed from the thermostat, stoppered and weighed.

The important part of the apparatus is the tip on which the drops are formed. In the present experiments a glass tip was used. This was prepared as suggested by Harkins by inserting a piece of glass capillary tubing in a lathe and running the latter at high speed. A flat plate, on which was placed fine wet carborundum powder, was held against the side of the rotating glass and the side ground until the glass had a uniformly circular cross section. The side was then carefully polished and the end of the tube ground flat and perpendicular to the side. When ready for use no flaws in the circular edge of the tip could be detected on examination in a low power microscope.

At temperatures above 0° C. water was used as the thermostat liquid; at lower temperatures this was replaced by a mixture of snow and calcium chloride.

The carbon tetrachloride was the best obtainable commercially and was further purified. It was washed several times with pure sulphuric acid and then with distilled water. The washing was repeated with a solution of sodium hydroxide and finally with distilled water once more. Calcium chloride was then added to the tetrachloride and the container left in a shaker for 24 hr. in order to remove most of the water. The drying process was completed by the addition of pure sodium. Finally the liquid was filtered and distilled, the middle fraction alone of the distillate being used. This portion was redistilled and only the tetrachloride distilling off within 0.02°C. of the true boiling point was used in the experiments.

If the empty weighing bottle is fitted over the dropping tip at room temperature and the apparatus is then immersed in a thermostat at a lower temperature, there will be an appreciable condensation of vapor in the bottle. Consequently the mass of liquid found in the bottle at the end of the experiment will be the sum of the masses of the fallen drops and that of the condensation. The true drop weight can therefore not be determined directly from the weight of the liquid.

The correction for the condensation clearly will depend both on the initial temperature of the apparatus and also on the temperature of the thermostat. The initial temperature of the apparatus was kept constant in all the experiments by immersing the water-tight container, containing the weighing bottle and tip, in a water bath at 25° C. The apparatus remained in this bath until temperature equilibrium was established and was then transferred to the cold thermostat for the experiment proper. In order to eliminate the varying effect of the vapor condensation at different thermostat temperatures the experiment was performed in two portions. In the first, the mass (m_1 gm.)

of five drops plus the condensation was determined; in the second, the mass (m_2 gm.) of 30 drops plus the condensation was obtained. Subtraction of the two results at any given thermostat temperature then gives the true weight of 25 drops at this temperature. In practice, the large heat capacity of the water-tight container and its contents made it rather difficult to reproduce the lower temperatures exactly for a whole series of experiments. For this reason the mass m_1 was measured at a large number of different temperatures and the results represented graphically. A second similar series of experiments gave m_2, the mass of 30 drops plus the condensation, as a function of the temperature. From the two curves so obtained, the true mass of 25 drops could be read off at any desired temperature.

There are two difficulties peculiar to work at temperatures below room temperature. As soon as the apparatus is taken from the thermostat, the weighing bottle is removed from the dropping tip and tightly stoppered to prevent the escape of vapor. The liquid inside is at the temperature of the thermostat, which is considerably below room temperature. Consequently there is an immediate deposit of water vapor on the outside of the weighing vessel when the temperature of the latter is below the dew point. This may seriously interfere with the weighings.

Again when the bottle is stoppered, the temperature of the liquid inside is low and its vapor pressure is small. As the liquid temperature rises, the total pressure inside becomes greater than atmospheric pressure and there is a tendency for vapor to leak out of the container. The weighing bottles used had ground glass stoppers which were carefully reground to give as perfect a fit as possible so as to minimize the effect of this loss of vapor. With these precautions, the leak was very steady and was barely large enough to be noticed in weighing.

It was further observed that the water vapor, condensed on the outside of the weighing bottle, evaporated again very rapidly once the temperature of the bottle approached that of the room and that about one and a half or two minutes after removing the bottle from the thermostat all trace of the water vapor had vanished from the bottle.

The bottles were therefore weighed in the following standard manner. After removal from the thermostat, the bottle was stoppered immediately and placed on a metal plate so that its temperature rose uniformly and fairly quickly to that of the room and the water vapor evaporated from its surface. The bottle was weighed exactly two minutes after it had been taken from the thermostat. By this time its outer surface was perfectly free from water so that the weight obtained was the true weight of the bottle and the contained liquid. Further, if the time between removal from the thermostat and the weighing of the bottle is the same in all cases, the small amount of carbon tetrachloride vapor lost by leakage will be very approximately the same for an experiment with five drops as for one with 30 drops, so that no appreciable error will appear in the drop weight deduced from the two curves. All the masses given in the graphs are therefore those of the liquid contained in the

weighing bottle two minutes after removal from the thermostat. It is belie that this procedure eliminates any error due to condensation on the out of the bottle.

Results

The radius of the dropping tip was measured by means of a low po microscope. The mean value of 20 measurements gives

$$R = 0.19726 \text{ cm.},$$

the average error of the measurements being 0.00019 cm.

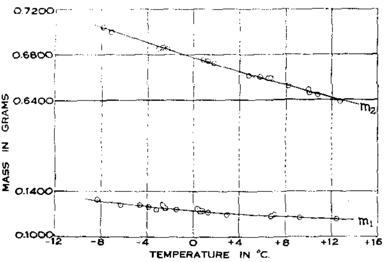

FIG. 1. *Variation of the masses m_1 and m_2 with temperature.*

The time of formatior a drop when allowed to fc freely on the tip was ab four minutes.

It will be seen from I 1 that the graphs of and m_2 as functions of temperature are very nea linear and over the sh range of temperature u in these experiments, results can be represen by an equation of the ty

$$m = a + bt + ct^2.$$

This equation was fitted to the experimental data by the method of le squares and gave for the mass of five drops plus the condensation

$$m_1 = 0.12352 - 8.3938 \times 10^{-4}t + 1.9168 \times 10^{-5}t^2$$

while for the mass of 30 drops plus condensation it gives

$$m_2 = 0.67755 - 31.533 \times 10^{-4}t + 1.7944 \times 10^{-5}t^2$$

In Fig. 1 the circles represent the actual experimental points while the cur drawn are those given by Equations (2) and (3).

Finally by subtraction of Equation (2) from Equation (3) we have the m of 25 drops and hence the mass (m gm.) of one drop in the form

$$m = 0.022161 - 9.2557 \times 10^{-6}t - 4.896 \times 10^{-8}t^2$$

The values of m have been calculated from Equation (4) at intervals of between +12° C. and −10° C. The surface tensions are deduced therefr by direct application of the equation

$$\gamma = \frac{mg}{2\pi R f\left(\frac{R}{V^{\frac{1}{3}}}\right)},$$

the correction factor $f\left(\frac{R}{V^{\frac{1}{3}}}\right)$ being obtained from Harkins' table.

The results of the calculation are given in Table I.

TABLE I

RESULTS OF CALCULATIONS

t, ° C.	m, gm.	Dynes per cm.	t, ° C.	m, gm.	Dynes per cm.
+12	.021043	27.80	+ 1	.022068	29.14
+11	.021137	27.92	0	.022161	29.27
+10	.021230	28.04	− 1	.022253	29.39
+ 9	.021324	28.17	− 2	.022346	29.51
+ 8	.021417	28.29	− 3	.022438	29.63
+ 7	.021506	28.41	− 4	.022532	29.75
+ 6	.021604	28.54	− 5	.022623	29.87
+ 5	.021697	28.66	− 6	.022714	29.99
+ 4	.021791	28.78	− 7	.022806	30.11
+ 3	.021883	28.90	− 8	.022898	30.24
+ 2	.021976	29.02	− 9	.022990	30.36
			−10	.023082	30.48

The only data with which the above measurements may be compared directly are those of Harkins and Cheng (3) who give values of 28.05 dynes per cm. at +10° C. and 29.38 dynes per cm. at 0° C. It will be seen that their value at +10° C. is practically identical with that given above, while the agreement at 0° C. is not quite so good. They do not give the details of the purification of their material and with the exception of the two values quoted, their measurements were all made at higher temperatures outside the range of the present experiments. Richards and Carver (5) using extremely pure carbon tetrachloride made accurate measurements of the surface tension at 20° C. This temperature is higher than any used in the present work, but it is interesting to note that extrapolation of the Equation (4) to 20° C. gives γ = 26.80 dynes per cm. which compares very closely with Richards' value of 26.77 dynes per cm.

Acknowledgment

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THE SOUND FIELD OF MEMBRANES AND DIAPHRAGMS

I. THE ANGULAR DISTRIBUTION OF THE SOUND PRODUCED BY A LARGE DIAPHRAGM[1]

By R. Ruedy[2]

Abstract

The amplitudes of the sound waves set up by a thin circular plate clamped at the edge and vibrating with circular and radial nodes, have been calculated for distances which are large with respect to the radius of the disk (loudspeaker). The results are compared with those obtained at various frequencies when a rigid oscillating piston set into an infinite wall is used as a source or reproducer of sound. The two main differences between the vibrating plate and piston are the much greater amplitudes obtained in certain directions when the diaphragm is used, even though the displacement at the centre of the disk may be the same in both cases, and the strong tendency to send the sound sideways instead of directly forward. While the diaphragm shares these properties to a certain extent with the stretched membrane, it displays them in a more practical range. The theoretical frequencies at which these features are found to be most pronounced are in agreement with the values found by experiment.

Introduction

When studying surfaces and materials which are used as reproducers or sources of sound, a short rigid oscillating piston set into an infinite wall is commonly chosen as the basis of comparison. For ordinary purposes the radius of the disk or flat cone is about a decimetre so that up to at least 250 cycles per sec., the point where the wave-length in air has dropped to 133 cm., the dimensions of the disk are small compared with the wave-length. Below this frequency, which comprises only the lowest range of the vibrations reproduced in the ordinary telephone talking circuit (250 to 3,000 cycles per sec.), the disk acts more or less like a point source giving divergent waves with, at a given distance, the same amplitude whatever the direction. Above this frequency, however, up to 10,000 cycles per sec., the highest frequency for which modern transmitting circuits are designed, interference effects appear, and the sound becomes more and more concentrated along a few narrow beams. Indeed, for short waves, the variations in the distances of a given point from the various elements of the vibrating surface are of the order of the wave-length itself and the impulses received differ in phase except in the cases of those points which lie far away upon lines parallel to the normal to the disk (12, 16–18).

The to-and-fro motion given to the reproducing surface—which is of the same type whatever the kind of sound concerned—approaches the behavior of the rigid piston. There are, however, not many musical instruments which approximate this artificial source (1).

On the other hand, in order to get, with moderate magnetic or electrical forces, amplitudes of practical value, it is necessary to keep the mass of the

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moving system small and the surface thin, this of course at the expense of stiffness. The rigid non-resonant piston thus becomes a thin plate, and the consequence is that at the higher frequencies all the disks and cones used in speakers vibrate in sections separated by circular or radial nodes. Recent investigations undertaken in various laboratories have actually established the fact that loudspeakers commonly possess fundamental natural frequencies of but a few hundred cycles per sec., and cease to be rigid above this point, that is, in the range where the focusing effect of the rigid disk begins (2, 3, 8–12, 19–22, 24). Moreover, in at least one type of loudspeaker, namely, in the electrostatic type of reproducer, a thin membrane is caused to vibrate in an extended alternating electric field in such a way that there no longer remains any resemblance to the rigid piston at any frequency (7, 13, 23). Even walls in buildings behave as large diaphragms when transmitting sound from one room to the other.

In view of these conditions the surfaces of loudspeakers may be expected to combine the properties of pistons and diaphragms, and it is of interest to determine what properties are possessed by the sound field created by diaphragms or thin plates and what is the power emitted, computing the values to the same degree of precision as has been attained with the rigid piston.

The Transverse Vibrations of Diaphragms (Thin Circular Plate Clamped at the Edge)

Among vibrating plane surfaces the diaphragm, a very thin circular plate clamped along the edge, as far as mathematical analysis is concerned, is the two-dimensional development of the thin rod in the same way that the drum-head or membrane is related to the string. Plates of appreciable thickness form the transitional stage between oscillating plane surfaces and solid bodies vibrating in three dimensions. Once the surface has been deformed and released, the stiffness of the thin plate suffices to bring it back into its former shape. The equations of motion of a surface element, under the uniform external force $N_0\epsilon^{i\omega t}$, are as follows (4, 6, 14, vol. I, p. 358):

$$\Delta^4 w + \frac{12s(1-\mu^2)}{Eh^2}\frac{\partial^2 w}{\partial t^2} = \frac{12(1-\mu^2)}{Eh^3} N_0\epsilon^{i\omega t} ,$$

where

$$\Delta^2 = \frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial^2}{\partial r} + \frac{1}{r^2}\frac{\partial^2}{\partial \phi^2} .$$

and w is the elongation normal to the surface, h is the thickness of the plate E the modulus of elasticity of the material, s the specific gravity and μ is Poisson's ratio for the material.

In the case of resonance ($N_0=0$) and assuming sinusoidal displacements, each element given by its polar coordinates (r, ϕ) performs a simple harmonic oscillation normal to the thin plate according to the law

$$w = \left[J_n(\kappa r) - \frac{J_n(\kappa a)}{J_n(i\kappa a)} J_n(i\kappa r)\right] (A_n \cos n\phi + B_n \sin n\phi)\, \epsilon^{ipt}$$

where

$$p = 2\pi f = \kappa^2 a^2 \frac{h}{2a^2}\sqrt{\frac{E}{3s(1-\mu^2)}}$$

for free or clamped plates when f is the resonance frequency of vibration. In the case of the membrane, the motion is given by the equation

$$w = J_n(\kappa r)\,(A_n \cos n\phi + B_n \sin n\phi)\epsilon^{ipt} \ .$$

Moreover, by virtue of the boundary conditions for the clamped plate, only those values of κ are admissible for which the Bessel functions J satisfy the relation

$$\frac{J_n(\kappa r)}{J_n(i\kappa r)} = \frac{dJ_n(\kappa r)/dr}{dJ_n(i\kappa r)/dr} \qquad \text{for } r = a, \text{ the radius of the disk,}$$

or

$$\frac{J_n(\kappa r)}{J_{n-1}(\kappa r)} = \frac{J_n(i\kappa r)}{iJ_{n-1}(i\kappa r)} \qquad \text{for } r = a.$$

A number of solutions $\kappa_{n0}a,\ \kappa_{n1}a,\ \kappa_{n2}a \ldots\ \kappa_{n\nu}a$, and therefore a number of resonance frequencies $f_{n\nu}$, result for each n, as shown by Table I, the overtones of the thin plate progressing more rapidly to higher values than those of the membrane and producing a somewhat more harmonious sound (Fig. 1). Neglecting μ^2 (which usually lies between 0 and 0.25) the resonance frequency may be written

$$f_{n\nu} = 0.046\,\frac{\kappa^2_{n\nu}a^2}{a^2}\,hC\,,$$

for instance, $f_{00} = 0.466\ hC_m/a^2_{dm}$, with C_m, the velocity of sound varying from about 500 m. for cork to 5,000 m. for aluminium and iron, and it follows that for a disk of 10 cm. radius, resonance is produced at short wavelengths unless the thickness of the plate be kept very small ($h < 0.1$ cm.). Interference effects are therefore to be expected as a rule in the space facing

TABLE I

SOLUTIONS OF THE DIAPHRAGM EQUATION $J_n(\kappa a)J'_n(i\kappa a) = J_n(i\kappa a)J'_n(\kappa a)$

	$\nu = 0$	$\nu = 1$	$\nu = 2$	$\nu = 3$	$\nu = 4$	ν
$n = 0$	3.1961	6.3064	9.4395	12.577	15.716	$(\nu + 1)\pi$
1	4.611	7.7993	10.958	14.108	—	$(\nu + 3/2)\pi$
2	5.9056	9.1967	12.402	15.579	—	$(\nu + 2)\pi$
3	7.143	10.537	13.795	—	—	$(\nu + 5/2)\pi$
n	—	—	—	—	—	$\left(\nu + 1 + \frac{n}{2}\right)\pi$

(*n = number of nodal diameters, ν number of nodal circles*).

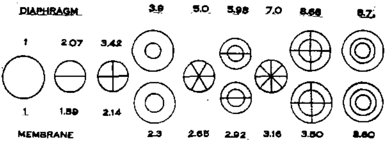

FIG. 1. *Overtones of diaphragm and membrane for similar modes of vibration (fundamental = 1).*

the vibrating thin plate clamped along the circumference and set into an infinite wall. They are liable to enhance the uneven distribution of sound which is caused by parts of the diaphragm vibrating in phase opposition as

compared to the rest of the surface. The number of nodal diameters is particularly important in this respect. When there is an even number of diameters upon which the elements remain permanently at rest, any two points symmetrically situated with regard to the centre move in exact phase. When the number is odd, the two symmetrical points have the same amplitude, but move in different directions.

From the equation giving the displacement of the surface elements the velocity of the points along the direction normal to the disk can be expressed as follows:

$$w' = \dot{w}(r, \phi)\epsilon^{ipt} = \left[J_n(\kappa r) - \frac{J_n(\kappa a)}{J_n(i\kappa a)} J_n(i\kappa r)\right] (A'_n \cos n\phi + B'_n \sin n\phi)\epsilon^{ipt}$$

where $A'_n = ipA_n$ and $B'_n = ipB_n$.

Pressure Amplitude and Direction

The problem is to compute for the distant point P the pressure p of the air and the velocity $\dot{v}$ resulting from the vibrations of the diaphragm set into an infinite wall. The shortest method is first to ascertain Rayleigh's velocity potential Φ at the point selected:

$$\Phi = -\frac{1}{2\pi}\int_S \dot{w}(r, \phi)\, \epsilon^{ipt} \frac{\epsilon^{-ikR}}{R} dS \ ,$$

where R is the distance of the point P from the various surface elements, and $k = 2\pi/\lambda$. In the present case, point P is chosen so far away that the lines connecting it with the surface are all parallel to each other.

The centre of the circular diaphragm is taken as the origin of a system of polar co-ordinates (Fig. 2). A perpendicular from the surface element dS at the point $S(r, \phi)$ upon the line OP joining the distant point $P(r, \phi, y)$ to the origin intersects OP at the distance l from the origin. If the distance OP is made equal to R_0, then PS equals $R_0 - l$, and the distance from the point S' symmetrically situated with respect to the origin is equal to $R_0 + l$ where, with ρ as the angle between OS and OP,

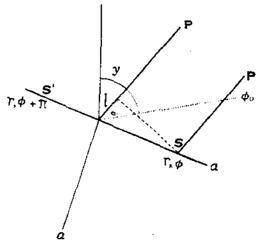

FIG. 2. *Relation between surface element at $S(r, \phi)$ and distant point P for the vibrating thin plate.*

$$l = r\cos\phi = r\cos(\phi_0 - \phi)\sin y \ .$$

Therefore

$$\Phi = -\epsilon^{ipt}\frac{\epsilon^{-ikR_0}}{2\pi R_0}\int_S \dot{w}(r, \phi)\,\epsilon^{\pm ikl} dS \ .$$

Here the value of R_0 is constant and much larger than l as long as we are interested only in the effects produced at remote points along a chosen direction.

When surface elements which lie symmetrically with respect to the centre vibrate with the same amplitude and phase, that is, when $w(r, \phi)$ equals

$w(r, \phi+180)$, and n is even, corresponding pairs of points may be combined and give

$$\Phi = -\epsilon^{ipt}\frac{\epsilon^{-ikR_o}}{\pi R_o}\int_0^r\int_0^\pi r\,dr\,d\phi\,\dot{w}(r,\phi)\cos kl\ ,$$

and in the case of opposite phases (n an odd number)

$$\Phi = -i\epsilon^{ipt}\frac{\epsilon^{-ikR_o}}{\pi R_o}\int_0^r r\,dr\int_0^\pi \dot{w}(r,\phi)\sin(kr\sin y\cos(\phi_o-\phi))\,d\phi\ ,$$

where in both cases

$$\dot{w} = \left[J_n(\kappa r) - \frac{J_{n\nu}(\kappa a)}{J_{n\nu}(i\kappa a)}J_n(i\kappa r)\right]\left[A'_{n\nu}\cos n\phi + B'_{n\nu}\sin n\phi\right]$$

Pressure P and velocity v at the point (r, ϕ) follow from the relations $P = -\rho\partial\Phi/\partial t$ (where ρ is the density of the air) and $v = -\partial\phi/\partial R$, giving a velocity component in phase and a component out of phase with the pressure which for even values of n are:

$$v = \frac{ik}{\pi R_o}\epsilon^{ipt}\epsilon^{-ikR_o}\int_0^r\int_0^\pi r\,dr\,d\phi\,\dot{w}(r,\phi)\cos kl - \frac{1}{\pi R_o^2}\epsilon^{ipt}\epsilon^{-ikR_o}\int_0^r\int_0^\pi r\,dr\,d\phi\,\dot{w}(r,\phi)\cos kl.$$

For computing the integral entering into the velocity potential Φ, advantage may be taken of the following relations:

$$\cos(g\cos\phi) = J_o(g) - 2J_2(g)\cos 2\phi + 2J_4(g)\cos 4\phi - \ldots.$$

$$\sin(g\cos\phi) = 2J_1(g)\cos\phi - 2J_3(g)\cos 3\phi + 2J_5(g)\cos 5\phi\ \ldots$$

and as

$$\int_0^\pi \cos n\phi\cos(g\cos\phi)\,d\phi = \pi J_n(g),\text{ if } n \text{ is even;}\quad \text{zero, if } n \text{ is odd.}$$

$$\int_0^\pi \sin n\phi\sin(g\cos\phi)\,d\phi = \text{zero, if } n \text{ is even;}\quad \pi J_n(g),\text{ if } n \text{ is odd,}$$

hence, by using $(\phi_o - \phi)$ as the new variable:

$$\int_0^\pi \cos n\phi\cos(g\cos(\phi_o-\phi))\,d\phi = \pi\cos n\phi_o\,J_n(g),\text{ if } n \text{ is even,}$$

$$\int_0^\pi \sin n\phi\sin(g\cos(\phi_o-\phi))\,d\phi = \pi\sin n\phi_o\,J_n(g),\text{ if } n \text{ is odd,}$$

$$\int_0^\pi \sin n\phi\cos(g\cos(\phi_o-\phi))\,d\phi = \text{zero, if } n \text{ is even,}$$

$$\int_0^\pi \cos n\phi\sin(g\cos(\phi_o-\phi))\,d\phi = \text{zero, if } n \text{ is odd.}$$

Finally

$$\pi\int_0^{r=a} dr\, r\, J_n(\kappa r) J_n(kr \sin y)$$
$$=\frac{\pi a^2}{\kappa^2 a^2-(ka \sin y)^2}\,(ka \sin y\, J_{n-1}(ka \sin y)\, J_n(\kappa a) - \kappa a\, J_{n-1}(\kappa a)\, J_n(ka \sin y))$$

$$\pi\int_0^{r=a} dr\, r\, J_n(i\kappa r)\, J_n(kr \sin y)$$
$$=\frac{-\pi a^2}{\kappa^2 a^2+(ka \sin y)^2}\,(ka \sin y\, J_{n-1}(ka \sin y)\, J_n(i\kappa a) - i\kappa a\, J_{n-1}(i\kappa a)\, J_n(ka \sin y))$$
$$=\frac{-\pi a^2}{\kappa^2 a^2+(ka \sin y)^2}\,\frac{J_n(i\kappa a)}{J_n(\kappa a)}\,(ka \sin y\, J_{n-1}(ka \sin y)\, J_n(\kappa a) - \kappa a J_{n-1}(\kappa a)\, J_n(ka \sin y)).$$

With the two terms combined and taking into account the boundary condition the final result of the integration, apart from the factor

$$-\,2\kappa^2 a^2\, a^2 \epsilon^{-ikR_o}\, \epsilon^{ipt} A'_{n\nu}/R_o$$

is given by

$$\Phi \parallel \cos n\phi_o \frac{ka \sin y\, J_{n-1}(ka \sin y)\, J_n(\kappa_{n\nu} a) - \kappa_{n\nu} a\, J_{n-1}(\kappa_{n\nu} a)\, J_n(ka \sin y)}{(\kappa_{n\nu} a)^4-(ka \sin y)^4}$$

for even values of n, and by the same expression except that the cosine is replaced by the sine function and A by B for odd values of n.

This result may be compared with the expression valid for the clamped circular membrane for which $J_n(\kappa a) = 0$, and

$$\Phi \parallel \cos n\phi_o \frac{\kappa_{ns} a\, J_{n-1}(\kappa_{ns} a)\, J_n(ka \sin y)}{(\kappa_{ns} a)^2 - (ka \sin y)^2}$$

for even values of n, apart from the factor $-a^2\epsilon^{-ikR_o}\epsilon^{ipt}A'_{ns}/R_o$, and, finally, with

$$\Phi = -\, a^2 \epsilon^{ipt} \frac{\epsilon^{-ikR_o}}{R_o}\, \dot{w}_o \frac{J_1(ka \sin y)}{ka \sin y},$$

for the rigid piston, where $\dot{w}_o$ is the velocity amplitude of the solid elements, the same over the entire surface of the piston. The pressure at any distant point is found from Φ by multiplying with the factor ip and the density ρ of the air.

Discussion of Results

For a diaphragm of a given material, provided only that its thickness is sufficiently small, the wave-length corresponding to the fundamental frequency f_{oo} will be much larger than the radius a; in other words, ka and $ka \sin y$ become negligible compared to $\kappa_{oo}a$. However, by virtue of the formula determining the natural frequencies

$$p_{n\nu} = 2\pi f_{n\nu} = \frac{2\pi c}{\lambda_{n\nu}} = 0.29\,\frac{\kappa^2_{n\nu} a^2}{a^2}\, hC$$

(where c is the velocity of sound in air), the rate at which $k=2\pi/\lambda$ grows when progressively higher overtones are chosen is determined by the square of $\kappa_{n\nu}$ so that there exists a frequency at which $ka \sin y$ becomes equal to κa. Up to this stage, and for a given angle, the denominator of Φ has kept falling

steadily except in the neighborhood of the points for which the numerator reduces to zero, and the pressure amplitude at the point P has passed from negligible to large values. Beyond this point it drops again. The increase with frequency will be more rapid the larger the angle y.

The practical bearing which these results may have depend on the relation between k and κ:

$$k = \frac{2\pi}{\lambda} = 0.29\ \kappa^2_{n\nu}a^2 \frac{hC}{a^2c}.$$

Taking as an average value for C that of brass, namely, 3,580 m. per sec., or of oak or beechwood, 3,300 to 3,400 m. per sec., with the radius of the disk as before equal to 10 cm. and neglecting μ^2, then at resonance

$$k_{n\nu} = \frac{2\pi}{\lambda_{n\nu}} = 0.029\,(\kappa_{n\nu}a)^2\,h \longrightarrow 0.029\,\pi^2\left(\nu + 1 + \frac{n}{2}\right)^2 h$$

or

$$k_{n\nu} \longrightarrow 0.29\left(\nu + 1 + \frac{n}{2}\right)^2 h\ .$$

The corresponding values are shown in Table II.

TABLE II

VALUES OF $2\pi/\lambda$ AT THE RESONANCE FREQUENCIES FOR AN AVERAGE DIAPHRAGM

(C/c = 10 AND a = 10 cm.)

	Number of nodal circles				
	$\nu = 0$	$\nu = 1$	$\nu = 2$	$\nu = 3$	$\nu = 4$
$k_{0\nu}$	$0.295\,h$	$1.149\,h$	$2.573\,h$	$4.67\,h$	$7.03\,h$
$\kappa_{0\nu}a$	3.196	6.306	9.44	12.58	15.72
$k_{1\nu}$	$0.614\,h$	$1.758\,h$	$3.472\,h$	$5.75\,h$	
$\kappa_{1\nu}a$	4.61	7.8	10.96	14.11	
$k_{2\nu}$	$1.01\,h$	$2.4\,h$	$4.44\,h$	$7.02\,h$	
$\kappa_{2\nu}a$	5.91	9.12	12.40	15.58	
$k_{3\nu}$	$1.78\,h$				
$\kappa_{3\nu}a$	7.14				

For a given ν the values of ka and $\kappa_{n\nu}a$ become equal when n satisfies the relation $\left(\frac{n}{2}+\nu\right)ha \geqq 10$. For practical purposes ($h<0.1$ cm.) whenever the radius of the diaphragm exceeds several centimetres, the value of κa is always appreciably larger than $ka \sin y$ in the case of all the simpler forms of vibration (n or $\nu < 4$), and with all the common materials the great maximum of pressure amplitude is reached only in the ultrasonic region. With paper, however, resonance frequencies seem to be pronounced at $n>10$ (19). Below this value the amplitudes keep on increasing at each successive resonance frequency, the growth being most rapid for large angles with the normal.

In addition, there is a variation of the sound pressure amplitude as the angle y with the normal is changed. For a given resonance frequency the

value of the numerator depends only on the value of $ka \sin y$, being, for $n=0$, for instance, equal to $-ka \sin y\, J_1(ka \sin y)\, J_o(\kappa a)+\kappa a\, J_1(\kappa a)\, J_o(ka \sin y)$, or varying between $\kappa_{o\nu}a\, J_1(\kappa_{o\nu}a)$ and $+\,\kappa_{o\nu}a\, J_1(\kappa_{o\nu}a)\, J_o(\kappa a)-ka\, J_o(\kappa_{o\nu}a)\, J_1(ka)$ as y changes from 0 to 90°. For the fundamental resonance frequency and the first overtones the denominator retains the same sign. Beyond κa or $ka \sin y$ equal to 2.4 in the case of J_o and 3.8 in the case of J_1, the Bessel functions change sign as the angle is varied, and as the terms grow at different rates they cancel for certain angles y. Thus, for the third overtone there is no change in pressure along the direction which forms an angle of about 40° with the normal; for the fourth overtone ($\kappa a=15.72$, $ka=7.03$) the silent zones lie between 20 and 30°, and between 50 and 60°; for the fifth overtone between 10 and 20, 30 and 40, and 60 and 70°. For progressively increasing values we may write

$$\kappa_{o\nu}a = (\nu+1)\,\pi \text{ and } \kappa_{1\nu}a = \left(\nu+\frac{3}{2}\right)\pi\,,$$

and

$$J_o(\kappa_{o\nu}a) = \frac{\sin(\nu+1)\,\pi + \cos(\nu+1)\,\pi}{\pi\sqrt{\nu+1}} = \pm\frac{1}{\pi\sqrt{\nu+1}}\,,$$

$$J_1(\kappa_{o\nu}a) = \frac{\sin(\nu+1)\,\pi - \cos(\nu-1)\,\pi}{\pi\sqrt{\nu+1}} = \mp\frac{1}{\pi\sqrt{\nu+1}}\,,$$

so that

$$\Phi_{o\nu} \,/\!/\, \frac{\pm\, ka \sin y\, J_1(ka \sin y) \mp (\nu+1)\pi\, J_o(ka \sin y)}{\pi\sqrt{\nu+1}\,((\kappa+1)^4\pi^4 - (ka \sin y)^4)} \cos n\phi\,,$$

with the pressure amplitude dropping to zero for all values for which $(\nu+1)\pi=ka \sin y\, J_1(ka \sin y)/J_o(ka \sin y)$, in which formula $ka \sin y$ varies between 0 and ka. We have then as many silent zones as there are zero values of $J_1(ka \sin y)$ below $J_1(ka)$, evidently fewer, the smaller a and h, rules which are already valid for overtones of low order.

Finally, owing to the presence of the factor $\cos n\phi_o$ (or $\sin n\phi_o$ when n is odd) in the formula, no sound pressure changes develop in the planes perpendicular to the diaphragm and passing through the nodal diameters, a conclusion which is in agreement with the experimental results. The larger the number of nodes passing through the centre, the smaller the intensity in the forward direction along the normal axis, because of the higher order of the Bessel function $J_n(ka \sin y)$ which must then be used.

Comparison with the Angular Distribution Produced by a Membrane or Piston

In order to give an illustration of the angular distribution of pressure amplitude produced by the diaphragm, the formula has been applied to a thin plate vibrating with zero, two or four circular nodes ($n=0$), assuming $a=10$ and $h=0.1$ cm. The corresponding resonance frequencies are therefore $f_{oo}\approx 150$, $f_{02}=1{,}340$ and $f_{04} \approx 3{,}740$ cycles per sec. (Figs. 3 and 4). Although the higher frequency is still far below that at which the highest possible maximum of pressure is obtainable (at $ka=\kappa a\approx 31$, or f about 166,000), the

pressure amplitudes exceed the values for the rigid piston at practically every large angle, provided, of course, that the velocity amplitude of the central point is the same in both cases.

The power emitted sideways is most remarkable, and in distinct contrast to the behavior of the rigid piston which tends to throw the sound directly forward. This point would seem to warrant further experimental tests. At a frequency of 3,740 cycles per sec. the sound emitted by a rigid piston of 10 cm. radius lies almost entirely within the angle $\psi = \sin^{-1} 2\times10^4/af \approx 30°$.

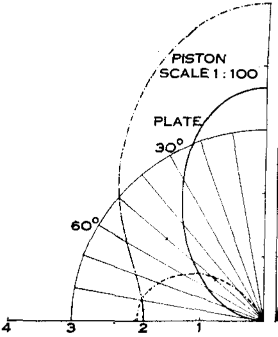

FIG. 3. *Polar diagram of the velocity potential Φ of a diaphragm (thin plate) vibrating with two circular nodes with piston for comparison. One unit of scale equals ϵ^{-ikR_0}/R_0. The lower curve represents the results for two diametral and one circular node and about the same resonance frequency. (1,350 cycles per sec.)*

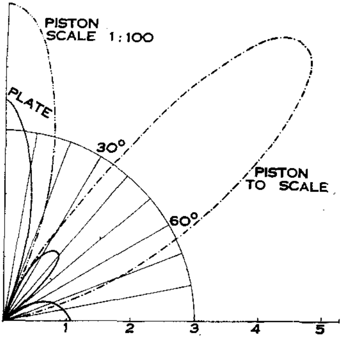

FIG. 4. *Polar diagram of velocity potential Φ of a diaphragm (thin plate) vibrating with four circular nodes, with piston for comparison. One unit of scale equals ϵ^{-ikR_0}/R_0. Resonance frequency, 3,750 cycles per sec.*

A second example corresponding to the vibration with two nodal diameters and in addition either no circular node ($f_{20} = 530$), or one circular node ($f_{21} = 1{,}260$) and three circular nodes ($f_{23} = 3{,}700$ cycles), or practically the same frequencies as before, shows that at all these frequencies there is little variation of the sound pressure amplitude when the angle with the normal increases from about 50 to 90°.

The advantages to be derived from the fact that a circular vibrating surface behaves as a flexible structure are, therefore, not only increased output, at certain resonance frequencies, but more even distribution of sound, even though the wave-length be smaller than the radius.

The thin circular plate clamped at the edge shares these properties to a certain extent with the clamped membrane. There exists, however, the difference that, owing to the relation,

$$2\pi f_{mn} = \frac{2\pi c}{\lambda_{mn}} = \kappa_{mn}\sqrt{\frac{T}{\sigma}}$$

or

$$k_{ns}a = \kappa_{ns}a\sqrt{\frac{T}{\sigma}} \quad ,$$

where κ_{ns} now is one of the solutions of the equation $J_n(\kappa a) = 0$, the values of ka and κa increase at the same rate, so that a stretched membrane either gives high sound pressure at all resonance frequencies (when $k = \kappa$), or, when the wave-length corresponding to the lowest natural frequency is larger than the diameter of the disk, negligible sound pressures at all frequencies.

Now the resonance frequencies f_{ns} of the membrane are given by

$$f_{ns} = \frac{\kappa_{ns}a}{2\pi a}\sqrt{\frac{T}{\sigma}}$$

where Tdl is the tension in dynes across a straight line of length dl drawn anywhere upon the membrane and σ the mass per sq. cm. A membrane sending out at resonance a wave-length comparable to its own dimensions (3,300 cycles per sec. or $\lambda = 10$ cm.) would require too high a tension to be safely applied to a membrane of 10 cm. radius, so that the high outputs are not obtained in practical cases when structures resembling clamped membranes are used as sources or reproducers of sound.

Another difference between membrane and diaphragm is the position of the silent zones. In the case of the circular membrane, no sound pressure develops along directions for which $J_n(ka \sin y) = 0$, or for a given frequency, along as many directions as there are nodal circles, the angles being, however, the same for a given n, apart from their number. For a diaphragm, on the other hand, the silent zones are given by (when $\kappa a \neq ka \sin y$)

$$\frac{J_n(ka \sin y)}{ka \sin y \, J_{n-1}(ka \sin y)} = \frac{J_n(\kappa_{n\nu}a)}{\kappa_{n\nu}a \, J_{n-1}(\kappa_{n\nu}a)} \quad ,$$

a relation more difficult to satisfy than the condition found for the membrane, and giving a smaller number of silent zones when n is large.

The results obtained have been used for computing the total power radiated by membranes and diaphragms. The results for very strong vibrations will have to be corrected by terms taking into account air-damping and radiation resistance.

Acknowledgment

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PYROLYSIS OF THE LOWER PARAFFINS

V. THE CONVERSION OF THE GASEOUS PARAFFINS TO AROMATICS IN BAFFLED METAL TUBES AND THE CHEMICAL COMPOSITION OF THE PRODUCTS[1]

By ADRIEN CAMBRON[2] AND COLIN H. BAYLEY[3]

Abstract

The thermal conversion of propane to aromatics has been investigated by passing the gas through externally heated alloy steel tubes under conditions of turbulent flow. Recycling experiments carried out under pressure have shown that, when the gas flow is turbulent, high rates of conversion can be obtained at temperatures as low as 800–810° C. Heat resistant chromium-nickel alloys of the 18 : 8 type have been found unsuitable for this purpose owing to the catalytic formation of carbon. Yields of 23.3 lb. of light oil per 1,000 cu. ft. of propane put through were obtained at 800° C. together with 10 lb. of liquids boiling above 200° C. The composition of the liquids obtained in these experiments has been determined by fractionation and chemical methods. The light oil obtained under the above conditions contains about 64% benzene, 14% toluene, 7.8% styrene and small amounts of cyclopentadiene, xylenes and higher aromatics. The liquids boiling above 200° C. contain about 25% naphthalene and 12.5% anthracene together with smaller amounts of mono- and dimethyl naphthalenes, acenaphthene and phenanthrene.

A considerable number of investigations have been reported in the literature on the conversion of the higher gaseous paraffins to aromatics. In most of these investigations silica or porcelain tubes were used, but a few experimenters, in particular Zanetti (23), determined the effect of carrying out the thermal treatment of hydrocarbon gases in metal tubes, or in the presence of metals, such as copper, iron or nickel. The work done on the subject up to 1929 is comprehensively reviewed in a survey published by Hague and Wheeler (13), who included in their paper the results of their own experiments in silica and porcelain tubes, and their conclusions as to the probable mechanism of the formation of aromatics from gaseous paraffins at high temperatures. Chamberlin and Bloom (5) have investigated the use of copper, steel and Monel metal tubes in the production of aromatics from paraffins. Wheeler and Wood (21) noted the similarity between the results obtained when methane is heated to 1050° C. in silica or chrome-iron tubes for a given time

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of contact. These authors did not specify the composition of the alloy used. Later Podbielniak (18), using special alloy steel tubes, the composition of which was not given, reported the results obtained in the course of semi-commercial scale experiments on the conversion of stabilizer and refinery gases into aromatics. In both of the above investigations it was found that the deposition of carbon upon the metals used caused considerable difficulties. In Podbielniak's experiments, for instance, it was found necessary to "blow" the reaction tubes with steam every 8–12 hr. in order to remove the carbon deposit. More recently this author has stated* that nichrome appears to be a more suitable material than the alloys so far investigated for the thermal treatment of hydrocarbons. Porter (19) reports that iron, nickel and similar metals possess a harmful and catalytic influence in the production of benzol by the thermal treatment of refinery gases and are therefore not suitable materials from which to construct reaction tubes. It was found, however, that an iron tube coated with chromium or tin could be used satisfactorily at temperatures of 825–950° C. Harnsberger (14) found that chrome-nickel alloy steels were suitable at 700–760° C. in the production of oil gas by the pyrolysis of petroleum fractions. It was necessary, however, to "blow" the cracking tubes periodically with steam or air in order to remove the deposit of carbon.

Steigerwald (20) in a patent on the conversion of gases containing methane to liquids claims that iron and the special alloy steels, *e.g.*, V2A or WT2, are useless, since they have the effect of promoting the decomposition of the hydrocarbon into carbon black and hydrogen. It is further claimed that heat resistant materials coated with chromium or molybdenum can be used at temperatures of 800–1200° C. Tubes protected in this manner are claimed not to catalyze carbon formation to any greater extent than quartz or porcelain.

Dunstan (7) has reported yields of 23.09% total liquids and 11.65% (=1.6 gal.) of light oil per 1000 cu. ft. of gas put through, in the pyrolysis of propane at 850° C. and atmospheric pressure. Wright (22) states that tubes of the 25-30% chromium alloys are suitable for vapor phase cracking operations when the pressure does not exceed 50 lb. per sq. in. Two vapor phase cracking units using tubes of this alloy and operating at temperatures as high as 870° C. have been in successful operation for over two years.

Foster (8) states that 25-30% chromium alloy tubes have been in use for three years in a vapor phase cracking unit operating at a temperature of 870° C.

Groll (11) has reported the results of experiments in which it was found that copper, iron and nickel tubes were unsuitable for the thermal treatment of gaseous and liquid hydrocarbons as these metals catalyzed the formation of carbon. He reports, however, that chrome-nickel alloy steel tubes of the 18 : 8 type, such as KA2, did not catalyze carbon formation. This latter observation does not agree with conclusions based upon the results of experiments carried out in these laboratories under similar conditions and using chromium-nickel alloy steel tubes of the 18 : 8 type.

*See Reference (9, p. 72).

The commercial development of thermal processes for the production of aromatics by the pyrolysis of the gaseous paraffins or the cracking of liquid hydrocarbons has been considerably retarded on account of the low throughput obtainable in pyrolysis or vapor phase cracking units. This difficulty can be largely overcome by the use of specially designed reaction tubes in which a high degree of turbulence is induced in the gas flow through the reaction chamber. As a result of the marked increase in the rate of heat transfer from tube wall to gas and the more uniform temperature distribution within the reaction space, the writers have shown, in previous communications (2, 3, 4), that the throughput of a given size of reaction tube at a given temperature is almost doubled when the tube is used for the conversion of gaseous paraffins to olefines, and increased by about 50% when the tube is used for the conversion of gaseous paraffins to liquid hydrocarbons. This has made it possible to obtain high rates of conversion at temperatures considerably lower than is the case when open tubes are used.

The purpose of this investigation was to determine the behavior of the chromium-nickel and high chromium heat resistant alloys in the conversion of propane to aromatics. Propane was chosen because this hydrocarbon is readily available and also because the conditions required for its thermal treatment are approximately the same as those required for the thermal treatment of refinery or stabilizer gases.

Materials Used

The propane used in these experiments was obtained from the Carbide and Carbon Chemicals Corporation. The value of n in C_nH_{2n+2} as determined by slow combustion analysis was 2.98.

Apparatus

The apparatus used in all except the pressure experiments was essentially the same as previously described, the furnace being the same as that used in the conversion of paraffins to olefines in metal tubes (3) and the recycling apparatus similar to that employed in the conversion of paraffins to liquids in quartz (4).

Experimental

(A) *Recycling Experiments in KA2S Tubes*

The following experiments were carried out particularly to determine the amount of carbon deposited in a baffled KA2S tube when propane is thermally treated for maximum liquid production. The results obtained are given in Table I. In this and subsequent tables, the letters used as column headings refer to the following data:—

A, total yield of liquids in gm. per hour;

B, percentage by weight of the propane put through recoverable as olefines from the exit gas;

C, percentage conversion to liquids, based on the weight of propane put through;

D, percentage conversion to light oil, based on the weight of propane put through;

E, yield of liquids in lb. per 1000 cu ft. propane put through;

F, yield of light oil in lb. per 1000 cu ft. propane put through;

G, ratio light-oil/tar;

H, carbon formed, gm. per hour.

TABLE I

EXPERIMENTS WITH KA2S AND 28% CHROMIUM NICKEL-FREE BAFFLES (KA2S TUBE)

Expt. no.	Temp., °C.	Gas rate, l/hr.		Duration of run, hr.	Expansion, %	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H**
		In	Recycle										
Baffles of KA2S													
37	917	143	340	1	101	54.6	30.3	19.6	12.9	23.9	15.6	2.0	20.0
38	920	144	289	1	103	68.2	26.9	24.3	15.6	29.6	19.0	1.8	8.0
39	922	144	279	1	100	66.3	—	23.6	14.3	28.8	17.4	1.5	7.0
40	900	143	239	5	99	56.6	31.0	20.3	13.1	24.8	16.0	1.8	7.2
41	900	143	243	2	104	57.4	32.3	20.6	13.7	25.1	16.7	2.0	6.3
Baffles of 28% chromium nickel-free alloys													
42	900	138.5	162	2	103	69.4	—	25.6	14.4	31.3	17.4	1.8	3.1
43	900	139.5	278	2	106	72.0	26.8	26.4	16.8	32.3	20.4	1.6	5.5

*Carbon removed by scraping.

It will be observed that the amount of carbon deposited per hour dropped considerably after the first experiment, but is, nevertheless, too great to permit the use of 18 : 8 chrome-nickel alloys under the above conditions. It was hoped that, with prolonged use, the activity of the metal would decrease. The above and following experiments show that this effect does not occur, the metal apparently retaining its activity even after 25-30 hours' use. In Experiment 40 the length of time the KA2S baffled tube could be used, before blocking due to deposition of carbon occurred, was found to be about five hours. The necessity of "blowing" the tube with steam or air at frequent intervals, in order to remove the deposit of carbon, makes it doubtful whether the use of this alloy would be practicable.

In Experiments 42 and 43 in which the KA2S baffles were replaced by baffles of 28% chromium nickel-free alloy the carbon formed decreased somewhat, but the difference is too small to permit any conclusions being drawn as to the relative effects of the two alloys.

It was found that the temperature required for the conversion of propane to liquids is appreciably lower in alloy steel tubes than in quartz tubes on account of the higher rate of heat transfer through the metal. Thus in previous

experiments in silica tubes (*cf.* Experiment 33 (4)) it was found that a conversion of 23.8% to liquids was obtained at 942° C., whilst in Experiments 42 and 43 in a metal tube of the same reaction volume, 25.6 and 26.4% conversion were obtained at 900° C.

The analyses of the gaseous products obtained in the above experiments are given in Table II.

TABLE II

ANALYSIS OF GASEOUS PRODUCTS. EXPERIMENTS 37-43

Expt. no.	% by volume			
	C_2H_2	C_2H_4	C_2H_6	H_2
37	2.2	19.0	2.0	29.6
38	2.6	16.8	1.7	31.9
40	2.0	20.3	1.6	28.2
41	2.1	20.6	1.7	28.1
43	2.6	16.6	1.6	31.6

The following series of experiments was carried out to determine whether the catalytic influence of the nickel alloy on carbon formation decreased after prolonged use. The results, which are given in Table III, show that it is very doubtful whether this material would prove suitable for the thermal treatment of paraffin gases as there was no evidence that the catalytic activity of the alloy decreased.

TABLE III

CARBON FORMATION WITH KA2S AND 28% CHROMIUM NICKEL-FREE BAFFLES (KA2S TUBE). TEMP., 900° C.

Expt. no.	Rate, l/hr.		Expansion, %	Duration of run, hr.	Carbon formed, gm.
	In	Recycle			
44	139.5	227	109.5	1	6.1
45	139.5	227	109.5	1	18.0
46	65.0	122	85.0	1	8.5
47	139.0	113.5	121.0	2	8.2
48	104.0	114	94.0	2	2.9
49	141.5	237	97.0	1	5.3
50	139.0	278	98.5	2	8.0
51*	140.0	278	98.0	1	15.0
52*	141.0	278	100.0	1	2.5
53*	139.0	287	95.0	2	0.9

**Baffles of 28% chromium nickel-free alloy.*

Although the above experiments were carried out under apparently similar conditions, for some unknown reason considerable variations were observed in the amounts of carbon formed. The results show, however, that the metal does not lose its catalytic activity with prolonged use.

In the experiments with nickel-free baffles (Experiments 51–53) the carbon was found to deposit almost exclusively on the wall of the tube, the only deposit found on the baffles being a very thin film of the lustrous grey carbon similar to that which forms on an inert surface, such as silica, under similar conditions.

The deposit of fluffy, black, catalytic carbon which was observed to form when the chromium-nickel alloy was used was thought to be due to the presence of nickel in the alloy. This was apparently confirmed when extraction of the carbon with hydrochloric acid gave a solution containing considerable amounts of nickel. The solution was also found to contain iron, but the presence of the latter element was not thought to have any marked effect on carbon deposition until it was observed that the formation of a voluminous deposit of catalytic carbon will also occur in nickel-free alloy tubes containing 20–30% chromium, if these are subjected to pyrolysis conditions for some time, then heated in air at 750–800° C. for removing the carbon film on the surface, and then used again for pyrolysis. When first used for pyrolysis, the surface of the high chromium alloy steel tubes and baffles becomes covered with a hard, gray film of carbon, which grows very slowly. If a tube and baffles are used for pyrolysis at 800° C. for several days, and then air passed through the tube at 750–800° C. for burning off the carbon film, the surface of the alloy becomes covered with a loose, brick-red deposit of oxides, which contain chromium as well as iron. There seems to be little doubt that the formation of this oxide film is responsible for the catalytic activity of the tube in promoting carbon deposition. Similar results have been obtained with KA2S alloy; heating in air after using tubes and baffles of this alloy for pyrolysis at 800–850° C. also results in the formation of a loose oxide film on the surface of the metal, and causes a considerable increase in the rate of carbon deposition under given conditions when the tubes are used again for pyrolysis. Groll (12) has also observed increased carbon deposition in KA2 and high chromium alloy tubes which had been heated in air after being subjected to pyrolysis conditions.

It may be pointed out that the use of baffles in the writers' experiments has made it very convenient to observe the condition of the surface of the metal after each experiment. It appears quite probable that the loss of resistance of the high chromium and of the 18–8 alloys against air oxidation is due to carburization of the alloy under pyrolysis conditions. This conclusion is based on the fact that the iron-chromium ratio of the oxide film in the case of the high chromium alloy is different from that of the alloy itself, and the observation that with the high chromium, as well as with the 18–8 alloy, if direct contact of the metal with the carbon film (which always deposits on the reaction tube and on the baffles during pyrolysis) is prevented by first covering the metal with an inert protective coating, these alloys can be repeatedly used for pyrolysis and heated in air without any oxide film forming on the surface, and without any apparent increase in the rate of carbon deposition.

The following experiments were carried out to determine the effect of increasing the recycle rate and the temperature on the yield of liquids and also to determine the effect of continued use on the deposition of carbon in the KA2S tube. In all of these experiments a tube of KA2S alloy with baffles of 28% chromium nickel-free alloy was used. The results obtained are given in Table IV.

TABLE IV

EXPERIMENTS AT HIGHER TEMPERATURE AND RECYCLE RATE WITH KA2S AND 28% CHROMIUM NICKEL-FREE BAFFLES (KA2S TUBE)

Expt. no.	Temp., °C.	Rate, l/hr.		Expansion, %	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
		In	Recycle									
54	900	139	411	98	66.9	28.8	24.7	18.3	30.1	22.3	2.9	2.5
55	903	142	546	91.3	49.5	—	17.8	14.5	21.7	17.7	4.5	7.6
56	930	142	546	109	71.0	28.4	25.6	18.7	31.2	22.8	2.7	2.6
57	902	192	427	101	81.7	34.4	21.8	16.3	26.6	19.9	2.9	2.9
58	930	192	448	108	102.7	30.9	27.4	19.1	33.4	23.3	2.3	3.8
59	930	214	377	110	108.5	29.2	25.8	17.3	31.5	21.1	2.0	2.4
60	930	187	450	120	100.1	—	27.4	18.5	33.4	22.6	2.1	2.3

It will be observed that in Experiments 54 and 55, in which the recycle rates were higher than those used in Experiments 40 and 41, the temperature being the same in both cases, the yields of light oil increased, whilst the light-oil/tar ratio was consistently higher, as compared with Experiments 41 and 42. In Experiment 56, in which the temperature was increased to 930° C., the recycle rate being the same as in Experiment 55, the yields of total liquids and light oil and the light-oil/tar ratio were the same as in Experiment 54, in which the temperature used was 900° C. and the recycle rate 411 litres per hour.

In Experiments 57-60, the inlet gas rate was increased, the temperature being raised to 930° C. in Experiments 58-60 and the recycle rate varied between 377 and 450 litres per hour. The optimum conditions appear to be those of Experiments 58 and 60, which gave a yield of total liquids of over 100 gm. per hr., the yield of light oil being equivalent to 23.3 lb. (2.7 gal.) per 1000 cu. ft. of propane put through. It will also be observed that the light-oil/tar ratio of the liquids obtained at the higher recycle rate is appreciably higher than in the previous experiments at lower recycle rates and lower temperature. The improved results obtained on increasing the recycle rate are undoubtedly due in part to the increasing effect of turbulence at the higher gas velocities.

TABLE V

ANALYSES OF GASEOUS PRODUCTS. EXPERIMENTS 54-59

Expt. no.	% by volume					*n* for residue
	C_2H_2	C_2H_4	C_3H_6	H_2	Residue	
54	2.2	19.5	1.2	29.4	47.7	1.03
56	2.7	18.6	0.8	31.1	46.8	1.01
57	2.3	21.9	2.1	27.1	46.6	1.00
58	2.9	17.2	2.9	30.5	47.5	1.07
59	2.9	17.6	1.8	31.1	46.6	1.02

The analyses of the gaseous products obtained are given in Table V.

(B) *Recycling Experiments in 28% Chromium Nickel-free Alloy Tubes*

In the following experiments the pyrolysis of propane to liquids was carried out in a tube of nickel-free 28% chromium alloy in order to compare the

behavior of this alloy, as regards carbon formation, with the KA2S alloy previously used. Owing to the relatively high creep rate of the 28% chromium alloy at temperatures above 850° C. the temperature was kept at or below this point. The results are given in Table VI.

TABLE VI

EXPERIMENTS WITH TUBE AND BAFFLES OF 28% CHROMIUM NICKEL-FREE ALLOY

Expt. no.	Temp., ° C.	Rate, l/hr. In	Rate, l/hr. Re-cycle	Expansion, %	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Pyrolysis in 85-cm. furnace											
61	835	219	232	87.0	32.4	49.0	7.0	5.6	9.2	6.8	—
62	840	211	237	98.5	41.3	53.9	11.0	8.4	12.2	10.2	—
63	850	212	230	98.5	47.6	46.8	11.5	9.1	14.0	11.1	3.7
64	833	208	228	94.0	37.1	49.2	9.1	—	11.0	—	—
Pyrolysis in 140-cm. furnace											
65	810	210	220	95.5	39.3	45.8	9.6	8.5	11.7	10.4	8.0
66	830	210	234	97.5	63.0	41.2	14.1	10.2	17.2	12.4	4.6
67	850	204	238	106	80.7	38.4	20.2	15.4	24.6	18.8	3.2
68	850	153	323	105	65.7	34.0	21.4	16.0	26.2	19.6	2.7
69	850	280	134	109	114.5	39.8	20.9	—	25.5	—	—

The analyses of the gaseous products from these experiments are given in Table VII.

TABLE VII

ANALYSES OF GASEOUS PRODUCTS. EXPERIMENTS 61-69

Expt. *no.*	% by volume C_2H_2	C_2H_4	C_3H_6	H_2	Residue	*n* for *residue*
61	0.6	26.7	7.7	19.8	45.2	1.20
62	1.0	27.6	8.0	19.5	43.9	1.16
63	1.7	29.0	3.6	22.2	43.5	1.03
64	0.0	27.9	6.0	18.8	47.3	1.20
65	0.7	24.8	3.4	18.3	52.8	—
66	0.7	24.0	4.3	20.2	50.8	—
67	1.4	24.7	1.7	22.2	50.0	1.00
68	1.3	22.5	1.1	24.1	51.0	—
69	0.4	25.3	1.7	25.4	47.2	—

In these experiments the amount of carbon deposited was practically negligible. A thin film of the hard, gray variety of carbon deposited on the tube wall and baffles. The rate of growth of the film, as determined by measuring its thickness after the reaction tube had been used for several hours, appears to be of the order of 0.007 mm. per hour at 850° C.

In Experiments 61–64 in which the length of the furnace was 85 cm. the maximum yield of liquids was 11.5% at 850° C. In order to increase the percentage conversion to liquids without raising the temperature, the heated length of the furnace was increased to 140 cm. by adding another section. The maximum percentage conversion of propane to liquids in the longer furnace was over 20% at a temperature of 850° C.

The yields of liquid and the throughput of the 140 cm. furnace could be increased by operating at a higher temperature or by further increasing the length of the furnace. Several limiting factors, however, prevented the carrying out of these experiments under more favorable conditions. The appreciable creep rate of the alloy used, when the latter is subjected to a temperature above 850° C., did not permit raising the temperature above that point, and made it impractical to increase the length of the furnace beyond the present dimension.

(C) *Recycling Experiments under Pressure in the 28% Chromium Alloy Tube*

The conversion of propane to liquids takes place in two distinct steps, (i) the cracking of the paraffin to olefines and (ii) the polymerization of the olefines to liquids. At atmospheric pressure and the temperatures used in the present experiments, the reaction involved in the first step takes place at a greater rate than the polymerization reaction. It was thought that increase

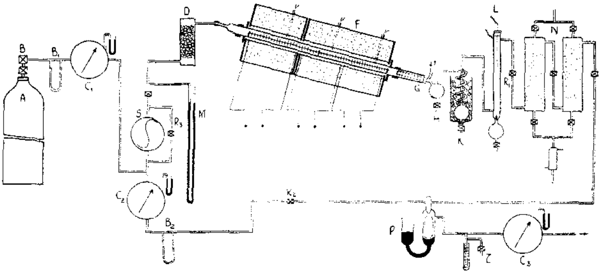

FIG. 1. *Apparatus used in pressure experiments.*

in pressure would, within limits, cause an increase in the rate of polymerization of the olefines without appreciably affecting the rate of cracking of the paraffin.

The following experiments were carried out to determine the effect of increasing the pressure up to one atmosphere on the conversion of propane to liquids.

The apparatus used is shown in Fig. 1. Gas was passed from the cylinder A through the low pressure valve B, the flowmeter B_1, the wetmeter C_1, the pump S, the calcium chloride tower D and the furnace F. The exit end of the reaction tube was cooled by means of the water cooled copper tube carrying baffles G. Most of the heavy tar in the exit gas separated out in H and a considerable amount of the lower boiling constituents collected in the condenser K, from which the gas passed to the Cottrell precipitator L and through the valve R_1 to the charcoal absorbers N which retained the butadiene, benzene and higher aromatics. The scrubbed gas then passed through the

three-way valve P from which a portion was recycled to the furnace through the flowmeter B_2, the wetmeter C_2 and the pump S, the remainder being vented to the hood through the wetmeter C_3. The pressure in the reaction chamber could be controlled by adjusting the valves R_1 and R_3. By adjusting the valve R_2, the desired fraction of the exit gas from the absorbers could be vented through the valve P or recycled through the pump S. The pressure of the gas in the reaction chamber was measured by the mercury manometer M. Samples of the vented gas were taken for analysis at Z.

All experiments were carried out in a 28% chromium alloy steel tube of 2.5 cm. internal diameter with baffles of the same material. The heated length of the tube was 85 cm. in Experiment 71 and 140 cm. in the other experiments. The results obtained are shown in Table VIII.

TABLE VIII

RECYCLE EXPERIMENTS AT PRESSURES HIGHER THAN ONE ATMOSPHERE

Expt. no.	Temp., °C.	Rate, l/hr.		Pressure, cm. Hg.	Expansion, %	A	B	C	D	E	F	G
		In	Recycle									
70	820	229	350	54	102	110.7	31.5	24.8	16.3	30.3	19.9	1.9
71*	810	232	334	47	96	97.2	34.0	21.4	17.0	26.2	20.8	3.7
72	800	229	388	74	98	125.2	27.1	28.0	19.1	34.2	23.3	2.1
73	800	276	413	72.5	97	111.5	—	20.7	16.8	25.3	20.5	4.4
74	810	276	387	73.5	99	127.4	33.2	23.6	17.4	28.8	21.2	2.8

*85 cm. furnace.

The analyses of the gaseous products obtained in certain of the above experiments are given in Table IX.

TABLE IX

ANALYSES OF GASEOUS PRODUCTS, EXPERIMENTS 70-74

Expt. no.	% by volume				
	C_2H_2	C_2H_4	C_2H_6	H_2	Residue
70	1.5	21.9	1.6	24.7	50.3
71	0.5	23.6	2.5	19.4	54.0
72	0.5	19.8	1.1	20.6	58.0
74	0.6	23.1	2.0	19.4	54.9

It will be observed that increasing the pressure up to about one atmosphere has a very marked effect on the rate of formation of liquids. Yields of light oil of over 20 lb. per 1000 cu. ft. were obtained in Experiments 72 and 74 at pressures of about 73 cm.

The effect of pressure is well illustrated by comparison of the results of the above experiments with those obtained when the pressure was atmospheric.

Thus, for instance, in Experiment 67 (Table VI) a yield of light oil of 18.8% was obtained at a temperature of 850° C., whilst in the above experiments yields of 23.3% at 800° C. and 21.2% at 810° C. were obtained although the inlet gas rate was appreciably higher. One would consequently expect

that the use of still higher pressures would cause a further reduction of the temperature required for the same rate of conversion, or, if the 28% chromium tubes can be used continuously at 800–810° C., an increase in the throughput of a reaction tube of a given size at that temperature.

It was expected that increase in pressure would tend to increase carbon deposition in the tube, but the present experiments have shown that there is no appreciable increase in the amount of carbon deposited when the pressure is increased to one atmosphere.

(D) *Composition of Gaseous and Liquid Products*

(1) *Gaseous Products*

Ethylene. It has been observed that when propane is thermally treated under what appear to be the optimum conditions for the production of aromatics, about 30% by weight of the propane is converted to olefines, mostly ethylene.

It is therefore obvious that the production of aromatics by the above process would have much greater commercial possibilities if the considerable amounts of olefines obtained as by-product could be profitably utilized for the production of such chemicals as ethyl alcohol or ethylene glycol. Under these conditions the total conversion to useful products (olefines plus aromatics) in the pressure experiments would be about 55%.

Butadiene. In steaming out the charcoal absorbers the vapors were passed through a condenser kept at 15° C. to condense the benzene and higher boiling constituents. The remaining gas which consisted mainly of butadiene, with smaller amounts of mono-olefines, was passed upwards through a water jacketed column filled with glass beads down which a 10% solution of bromine in carbon tetrachloride was passed. It was found that unless the solution of the bromides was allowed to stand for some time in contact with excess bromine, the conversion of the butadiene to the tetrabromide was not complete, considerable amounts of α, δ,-dibrombutene-2 being present. The solid and liquid isomers of this compound were separated in several experiments in which insufficient excess of bromine had been used.

The light oil recovered from the absorber contains some dissolved butadiene. In order to recover the butadiene dissolved in this oil, the latter was gently refluxed under a condenser cooled to 10-15°C. until the evolution of gas subsided, the evolved vapors brominated as before and the solution of bromides added to the main bulk which was then freed from excess bromine and dried. Most of the carbon tetrachloride was removed on the water bath and the residue fractionated under reduced pressure. The distillate up to 80°C./20 mm. was found to consist of ethylene and propylene dibromides. There was very little distillate between 80°C./20 mm. and 130°C./20 mm. At this point it was found that the residue in the flask partly solidified on cooling and on dissolving in hot 95% ethyl alcohol and allowing to cool, crystals of the high melting isomer of butadiene tetrabromide (m.p. 118°C. corr.) were deposited. The lower melting isomer was separated as a heavy oil from the residual

alcoholic solution by removing the alcohol *in vacuo*. The oil crystallized completely on cooling to 0° C. and after recrystallizing from 95% ethyl alcohol (in which the compound is very soluble) the crystals melted sharply at 37° C. The two isomers appeared to be produced in equal amounts which agrees with statements in the literature (10, 16). Since the tetrabromides were the only compounds remaining after fractionation of the mixed bromides to 130° C./20 mm., the amount of butadiene formed could be estimated accurately by weighing the tetrabromide residue.

Table X shows the yield of butadiene obtained in various experiments in which propane was thermally treated for the production of liquids in a tube of the 28% chromium alloy, and in which the butadiene formed was converted quantitatively to the tetrabromides.

TABLE X

YIELD OF BUTADIENE FROM PROPANE

Expt. no.	Temp., ° C.	Rate, l/hr.		Furnace dimensions	% C_4H_6, based on weight of C_3H_8 passed
		In	Recycle		
63	850	212	230	85 by 2.5 cm.	5.0
64	834	208	228	85 by 2.5 cm.	4.4
67	850	204	238	140 by 2.5 cm.	4.9

(2) *Light Oil (boiling below 200° C.)*

After the removal of butadiene and dissolved gases, the light oil was fractionally distilled in an electrically heated column filled with an efficient bronze gauze packing. Preliminary investigation showed that the main constituents of the light oil were cyclopentadiene, benzene, toluene, styrene and indene, along with small quantities of unsaturateds boiling up to 130° C., and possibly some tri- and tetramethylbenzenes. The curves obtained in fractionation of typical light oils obtained in the above experiments are given in Fig. 2.

It will be seen that the column employed gave sharp separation of the first three main constituents, the amounts of distillate obtained between the boiling points of these constituents being small. In the case of the styrene-xylene fraction however no sharp cuts could be obtained, but this fraction has been shown to consist largely of styrene.

The first or cyclopentadiene fraction was found to contain about 30% of cyclopentadiene together with smaller amounts of unsaturateds boiling up to 75° C., whilst the percentage of aromatics in the benzene and toluene fraction was over 95%. The styrene fraction has been shown to contain 70–80% of styrene whilst the remainder of the light oil distilling up to 200° C. contained about 70% of indene.

Cyclopentadiene fraction (25 to 78° C.). Cyclopentadiene was present in considerable amounts in the fraction boiling at 35–45° C. and was identified and gravimetrically determined by conversion to *cis*endomethylene 3,6-tetra-

hydrophthalic anhydride (m.p. 164.6° C. corr.) by reaction with maleic anhydride (1, 6). The fraction boiling between 45° and 75° C. was found to be highly unsaturated. The nature of the compounds present in this fraction was not investigated but it is probable that hexadienes and methylcyclopentadiene were present. In most cases the amount of cyclopentadiene in the total fraction was about 30% by weight. The presence of cyclopentadiene in the light oil obtained in the thermal treatment of the lower paraffins has been reported by Frey and Hepp (10), who also obtained indications of the presence of pentenes, hexadienes and methylcyclopentadiene in this fraction.

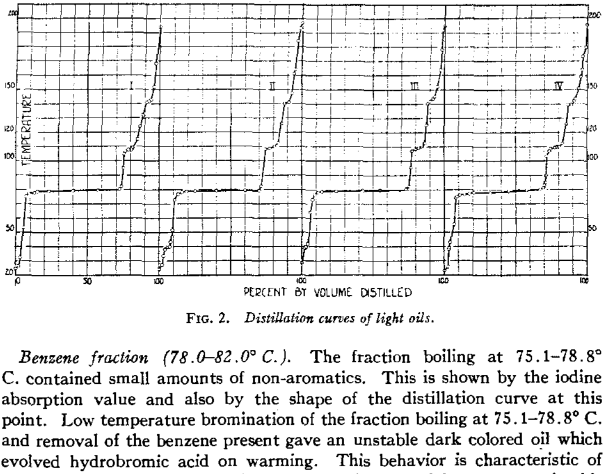

FIG. 2. *Distillation curves of light oils.*

Benzene fraction (78.0–82.0° C.). The fraction boiling at 75.1–78.8° C. contained small amounts of non-aromatics. This is shown by the iodine absorption value and also by the shape of the distillation curve at this point. Low temperature bromination of the fraction boiling at 75.1–78.8° C. and removal of the benzene present gave an unstable dark colored oil which evolved hydrobromic acid on warming. This behavior is characteristic of cyclohexene dibromide and it is likely that this material was present in this fraction since its presence has been reported in light oils of similar origin. The iodine number of this cut was not appreciably lowered by refluxing with maleic anhydride and distilling. Hence diolefines were probably absent. Assuming that the iodine absorption was due to the presence of cyclohexene, the amount of this material present would be 0.7% by weight of the total benzene fraction. These results show that the benzene fraction is practically free from gum forming constituents.

Table XI shows the distillation range, iodine absorption numbers (in centigrams of iodine per gram of material) and percentage of total oil distilling in the benzene range, the results being obtained on fractionation of a light oil produced by the pyrolysis of propane for the production of aromatics under the conditions described above.

Toluene fraction (82.0–126.0° C.). The cut boiling between 105 and 111° C. consisted chiefly of toluene which was identified by the preparation of the 2,4-dinitro derivative (m.p. 71.5° corr.) and comparison (mixed melting point) with an authentic specimen. The color of this fraction was pale yellow and the presence of a certain amount of unsaturation was shown by the iodine absorption values of the toluene fractions obtained from several samples of light oil. These together with the percentage yield of this fraction are given in Table XII.

TABLE XI

IODINE NUMBERS OF CUTS IN THE BENZENE FRACTION

Temp. range, ° C.	Iodine number, cgm. iodine/gm.	% by weight of cut based on total light oil
75.1–78.8	19.9	7.2
78.8–79.8	1.7	25.6
79.8–80.8	1.0	27.0
80.8–82.0	*2.5*	*11.2*

TABLE XII

IODINE NUMBERS AND YIELDS OF TOLUENE FRACTIONS

Expt. no.	Temp., ° C.	Rate, l./hr.		Furnace dimensions, cm.	Iodine no., cgm. iodine/gm.	% by weight of fraction based on total light oil
		In	Recycle			
	850	210	228.5	2.5 by 85*	14.7	11.5
65	810	210	220	2.5 by 140	11.4	13.1
68	850	153	323	2.5 by 140	11.4	11.3
71	*810*	232	*334†*	2.5 by *85*	*16.0*	13.9
72	800	229	388‡	2.5 by 140	13.2	11.1

**KA2S tube, all other experiments with 28% chromium alloy tube. †Pressure, 47 cm. Hg. ‡Pressure, 74 cm. Hg.*

Xylene fraction (126.0–164.0° C.). Most of the fraction boiling between 126 and 164° C. was found to distil in the range 135–145° C. When freshly distilled the color of this fraction is pale yellow, but darkens somewhat on exposure to light; on standing at room temperature for several weeks the fraction becomes exceedingly viscous. This fraction was highly unsaturated as shown by its high iodine absorption value. The presence of styrene in liquids obtained under similar conditions has been reported by other investigators (10, 11). Bromination of the xylene fraction of the light oil showed that it consisted mainly of styrene. There did not appear to be any other unsaturated compounds present in more than traces, since the residue of crude styrene dibromide had a melting point only slightly lower than that of the pure compound. After one recrystallization from 95% ethyl alcohol the dibromide melted at 73° C. (corr.). Hence it was possible to obtain a fairly accurate value for the amount of styrene present by means of the iodine absorption numbers and by direct bromination.

Owing to the importance of styrene as a raw material for the manufacture of synthetic resins, its presence in appreciable amounts in the light oil obtained

by the thermal treatment of propane is of interest. Table XIII shows the amounts of the fraction boiling at 126–164° C. as percentage of the total light oil, the percentage of styrene in that fraction and the yield of styrene based on propane put through. The styrene was determined by direct bromination and weighing the dibromide formed or by calculation from the iodine number. It will be seen that in most experiments the amount of styrene present was equal to over 70% of the total fraction.

TABLE XIII

YIELDS OF STYRENE IN THE PYROLYSIS OF PROPANE TO AROMATICS

Temp., ° C.	Rate, l./hr.		Furnace dimensions, cm.	Iodine no., cgm. iodine/gm.	Yield 126-164° C. fraction, % total light oil	% Styrene in 126-164° C. fraction	Yield styrene, as % propane passed
	In	Re-cycle					
840	211	237	2.5 by 85	175.9	7.9	72	0.58
850	210	228.5	2.5 by 85*	164.8	8.3	67	0.59
850	212	230	2.5 by 85	178.2	9.4	73	0.64
810	210	220	2.5 by 140	178.8	11.0	74	0.69
850	153	323	2.5 by 140	209	8.3	86	1.14
810	232	334†	2.5 by 140	183	7.7	75	1.00
800	229	388‡	2.5 by 140	197	6.6	80	1.01

**KA2S tube, all other experiments with 28% chromium alloy. †Pressure, 47 cm. Hg. ‡Pressure, 74 cm. Hg.*

On brominating a quantity of this fraction (50 gm.) collected in several experiments and distilling under reduced pressure, a small amount (6 gm.) of distillate was obtained which did not react with bromine and distilled in the range 130–145° C./760 mm. The amount was too small to permit an accurate fractionation being effected, but by distilling through a small bronze-packed column and collecting the portion distilling at 135–139° C., followed by strong nitration, *p*- and *m*-trinitroxylenes were obtained, the identities of which were confirmed by taking mixed melting points with authentic specimens.

Fraction boiling at 150–169° C. The small fraction obtained in this range was highly unsaturated. By applying the method used in separating the xylenes to a composite sample collected in several experiments, a few drops of liquid were obtained which on strong nitration gave a derivative having a constant melting point of 182° C. (corr.) after repeated recrystallization from 95% ethyl alcohol. This is only slightly below the recorded melting point of trinitropseudocumene and it is, therefore, likely that pseudocumene was present in small amounts.

Fraction boiling at 169–185° C. This fraction was also highly unsaturated, reacting with a well cooled solution of bromine in chloroform to give, on evaporation of the chloroform, a pale yellow oil that slowly crystallized on strong cooling. Indene was identified both by the properties of the dibromide

TABLE XIV

PERCENTAGE YIELDS OF MAIN FRACTIONS OF LIGHT OILS

Expt. no.	Temp., °C.	Rate, l./hr.		Furnace	Main fractions, % by wt. of light oil					Main fractions, % by wt. of propane passed				
		In	Recycle		Cyclo-pentadiene*	Benzene †	Toluene ‡	Styrene **	Indene ***	Cyclo-pentadiene	Benzene	Toluene	Styrene	Indene
58 59	930 930 †	192 214	448 377	85 by 2.5 cm., KA2S alloy	7.1	71.0	9.5	8.0	4.4	1.3	12.9	1.8	1.5	0.8
61	835	219	232	85 by 2.5 cm., 28% Cr alloy	22.3	47.4	13.2	11.6	5.5	1.2	2.8	0.7	0.6	0.3
62	840	211	237	85 by 2.5 cm., 28% Cr alloy	17.9	54.3	14.8	9.2	3.8	1.5	4.6	1.2	0.8	0.3
63	850	212	230	85 by 2.5 cm., 28% Cr alloy	11.8	55.8	15.3	11.4	5.7	1.1	5.1	1.4	1.0	0.5
65	810	210	220	140 by 2.5 cm., 28% Cr alloy	20.1	48.3	15.1	12.7	3.8	1.7	4.1	1.3	1.1	0.3
68	850	153	323	140 by 2.5 cm., 28% Cr alloy	9.7	62.4	12.3	12.5	3.1	1.5	10.0	2.0	2.0	0.5
71	810	232	334	140 by 2.5 cm., 28% Cr alloy. Pressure, 47 cm. Hg	11.9	57.6	14.3	10.5	5.7	2.0	9.8	2.4	1.8	1.0
72	800	229	388	140 by 2.5 cm., 28% Cr alloy. Pressure, 47 cm. Hg	8.6	65.1	12.3	10.8	3.2	0.6	12.4	2.4	2.1	0.6
74	810	276	387	140 by 2.5 cm., 28% Cr alloy. Pressure, 73.5 cm. Hg	6.1	63.0	15.7	11.4	3.8	1.1	10.9	2.7	2.0	0.7

***Composite sample fractionated.** †*Containing about 30% cyclopentadiene.* ‡*Aromatics above 95%.* ***Styrene 70-80%.* ****Indene about 70%.*

produced (m.p. 44° C.) and also by preparing the bromhydrin according to the method of Krämer and Spilker (15). These authors give the melting point of this compound as 130–131° C. (corr.?). The writers' compound after three recrystallizations from boiling water melted at 129.6° C. (corr.).

Estimation of the amount of indene in this fraction by direct bromination and weighing of the dibromide gave figures closely approximating those calculated from the iodine numbers. This would indicate that, except for traces of other olefines which were not identified, indene is the only unsaturated present.

Fraction boiling at 185–200° C. The amount of material distilling in this range was extremely small. Between 195–200° C. the distillate partly solidified on cooling. It possessed an odor resembling naphthalene, but it is not unlikely that higher methylated benzenes are present in small amounts. Beyond proving the presence of naphthalene in this fraction, its composition was not investigated further.

Table XIV shows the proportions of the main constituents for a series of light oils produced in the pyrolysis of propane in metal tubes. Experiments 61, 103, 104 and 105 were carried out mainly for the purpose of producing olefines with relatively low conversions to liquids.

(3) *Tar (boiling above 200° C.)*

Table XV shows the results obtained when a quantity of tar from the recycling experiments was fractionated under a pressure of 13 mm. in a 60-cm. bronze-packed column.

TABLE XV

RESULTS OF FRACTIONATION OF RECYCLE TAR

Temp., ° C./13 mm.	Main constituents	Yield, % by weight	
		Tar	Propane passed
65–97	Probably higher alkyl benzenes	4.5	0.4
97–113	Naphthalene	25.0	2.5
113–133	Methylnaphthalenes	3.1	0.3
133–146	{Dimethyl naphthalenes		
146–170	{Acenaphthene	6.5	0.7
170–186	Phenanthrene	5.0	0.5
186–215	Phenanthrene Anthracene	12.7	1.3
215–250	Probably methyl and dimethyl anthracenes	13.7	1.4
Above 250		29.5	2.9

Fraction boiling at 65–95° C./13 mm. This fraction was liquid at ordinary temperatures, but deposited a small amount of naphthalene on cooling to zero. Its composition was not investigated.

Naphthalene fraction; 97-113° C./13 mm. The fraction solidified completely on cooling and had a melting point only slightly lower than that of naphthalene. After one recrystallization from ethyl alcohol it melted at 80° C. and gave a picrate melting at 152° C. (corr.).

Methylnaphthalene fraction; 113–133° C./13 mm. On refractionation of the liquid fraction boiling at 113–133° C./13 mm. most of the fraction distilled at 235–245° C./760 mm. This suggests the presence of monomethylnaphthalenes. The fraction boiling at 239–242° C./760 mm., obtained on redistilling, solidified partly when cooled to −12° C. and gave a picrate melting at 116.5° C. which showed no depression of the melting point when mixed with an authentic sample of the picrate of the β-compound. The melting points of the picrates of the α- and β-compounds are 141° and 115° C. respectively. This would indicate that the fraction consists predominantly of the β-substituted methylnaphthalene.

In view of some recent work by Mayer and Schiffner (17), who found that α-methylnaphthalene was rapidly converted into the β-isomer over silica gel at 450° C., one may assume that, even in the absence of a catalyst, this reaction would be sufficiently rapid at 800–810° C. to convert any α-methylnaphthalene into the β-compound. This would explain why the α-methyl derivative, although undoubtedly formed under the conditions of these experiments, is not present in this fraction.

Dimethylnaphthalene fraction; 133–170° C./13 mm. This fraction gave a considerable amount of distillate at 260–270° C./760 mm. on refractionation. This yielded a picrate melting at 143.3° C. (corr.). On cooling to zero the fraction deposited crystals which were obtained as fine needles (m.p. 95.2° C. corr.) on recrystallization from ethyl alcohol. This compound was proved to be acenaphthene by the preparation of the picrate (m.p. 164.2° C. corr.) and of 4-bromoacenaphthene (m.p. 50° C. corr.) and by comparison (mixed melting points) with authentic samples of these compounds. The relative amounts of dimethylnaphthalenes and acenaphthene were not determined.

Phenanthrene-anthracene fraction; 170–215° C./13 mm. Repeated extraction of this fraction with hot ethyl alcohol gave a residue which crystallized in small leaflets from a large excess of ethyl alcohol and melted at 214° C. and which on oxidation with chromic acid gave anthraquinone (m.p. 285° C.). Phenanthrene was identified by treating an alcoholic extract of the fraction with picric acid. The picrate of anthracene is readily decomposed by alcohol, whereas that of phenanthrene is stable under these conditions. The picrate obtained melted at 144.2° C. and on decomposition with ammonia gave phenanthrene (m.p. 98° C.).

Fraction boiling above 215° C./13 mm. The fraction boiling at 217–232° C./13 mm. was a fluorescent liquid. At 234° C./13 mm. the distillate began to crystallize and the boiling point was constant at 234–235° C./13 mm. The temperature then rose rapidly to 244–246° C./13 mm., in which range a further quantity of distillate which solidified on cooling was obtained. These fractions were not investigated further.

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CHEMICAL INVESTIGATION OF THE CORMS OF *ARISAEMA TRIPHYLLUM* (L.) SCHOTT[1]

BY LÉO MARION[2]

Abstract

The dried and ground corms lost 5% of their weight when extracted with petroleum ether, and a further 0.9% on subsequent extraction with methanol. Besides a trace of an essential oil, the methanol extract yielded water-soluble and water-insoluble portions. The former contained an acid, $C_5H_8O_5$, m.p. 184° C.; *i*-inositol, $C_6H_6(OH)_6$, m.p. 224° C.; a sugar forming phenylglucosazone; and a crystalline substance, ($C_6H_{10}O_6$ or $C_7H_{12}O_7$), m.p. 120° C., exhibiting the properties of a lactone. In the water-insoluble portion combined with the petroleum ether extract, the following substances have been found present: myricyl alcohol, $C_{30}H_{62}O$, m.p. 82-83° C.; a new sterol, arisaesterol, $C_{36}H_{64}O_2$, m.p. 135° C.; phytosterolin, $C_{31}H_{52}O_6$, m.p. 297° C.; and a mixture of fatty acids.

Arisaema triphyllum (L.) Schott is a low perennial herb belonging to the family Araceae. It grows in eastern North America where it is known by the common name of Jack-in-the-pulpit, and although widely scattered, does not seem to occur very plentifully in any locality. Its tuberous root, or corm, contains an acrid juice which, when applied to the tongue causes, after a few minutes, a burning taste quickly becoming intolerable, and softening down gradually. It has been stated by Barnes (2) that this action on the mucous membrane is probably due to the rhaphides contained in the liquid, and that the juice after filtration had lost this property. But this seems doubtful, especially in view of the known fact that the corms, which are farinaceous, are used by the Indians as food; boiling in water or drying being reported sufficient to remove the acridity associating with the fresh plant, and render it edible.

Some work is extant on two species,—*Arum maculatum* L., and *Arum italicum* Mill. These seem to possess properties very similar to those exhibited by *A. triphyllum*. According to Hébert and Heim (4) *A. maculatum* contains a saponin in quantities seemingly not exceeding 1% of the weight of the fresh plant. This saponin, however, was not obtained in a crystalline condition nor studied beyond ascertaining that on hydrolysis, it yields an insoluble product and a reducing sngar (3). The juice permeating the corms is stated by the same authors to contain a liquid alkaloid closely related to coniceine, or to a homologous base. Although all parts of the plant are said to contain this alkaloid, the quantity present is extremely small (0.005% of the fresh plant). Practically the same has been recorded concerning *A. italicum* (7).

Owing to the interesting properties of *A. triphyllum* and the absence of information concerning it, together with the scanty nature of that relating to *A. maculatum* and *A. italicum*, it was deemed desirable to undertake the chemical investigation of the corms.

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It has not been possible to detect the presence of any alkaloid in the plant, nor of a saponin or any glycosidic product.

Efforts to detect another substance to which the acrid property of the fresh plant might be attributed also failed. That this property might be due to a very volatile product appears extremely doubtful; no evidence of the presence of such a substance was found on cautiously drying the ground corms at 60° C. It therefore seems more probable that the acridity might be due to some compound which is destroyed by heat.

Crystalline *i*-inositol $C_6H_6(OH)_6$, m.p. 224° C.*, was isolated in small amount. The plant extract has also yielded an acidic substance, $C_5H_8O_5$, m.p. 184° C., which, although isomeric, does not seem to be identical with the hydroxyglutaric acids. A further crystalline product having the composition and some of the properties of a sugar-acid lactone was also obtained, but it is neither *d*- nor *l*-allonolactone. This was ascertained by comparison with authentic specimens kindly supplied by Dr. P. A. Levene of the Rockefeller Institute, and Professor W. C. Austin, of the Loyola University School of Medicine, to whom the author acknowledges his indebtedness. Its composition agrees with $C_6H_{10}O_6$, although $C_7H_{12}O_7$ is not excluded.

Besides a sterol, a sterolin and myricyl alcohol, $C_{30}H_{62}O$, the extract was also found to contain a sugar which forms phenylglucosazone and various fatty acids. On account of the difficulty experienced in collecting sufficient material and the remarkably low percentage of the plant extracted by methanol, the quantities of the various products isolated were so small that a more detailed study was not possible. It is proposed to continue the investigation when more material becomes available.

Experimental

The material used in this investigation was collected in the Gatineau Hills and in the neighborhood of Mount Shepherd, in the province of Quebec. It consisted of the corms only, and, when dried, amounted to three kilograms. After washing rapidly with running water, the corms were sliced and dried in an oven at 60° C. in a blast of air. The dried product was then ground to a powder and extracted in Soxhlets, first with light petroleum and then with methanol. Petroleum ether removed 5.0%, methanol, 0.9%; the total extract being 5.9%. After removal of the greater part of the solvent from the methanolic extract, the residue was diluted with water.

Distillation of the Extract with Steam

The entire methanolic extract was subjected to distillation for several hours in a strong current of steam, and the distillate extracted with ether. After washing, drying and removal of the ether, the extract consisted of a small quantity of brown oil, but this was too small to allow further investigation.

**All melting points are corrected.*

Non-volatile Constituents of the Extract

After removal of the volatile constituents with steam, there remained in the distillation flask a dark-brown liquid *(A)* in which was suspended a quantity of dark, soft, resinous material *(B)*. The latter was separated by filtration through a layer of charcoal, washed with water and kept for further examination.

The Aqueous Liquid (A)

The aqueous liquid was reduced by distillation under diminished pressure to a volume of about one litre, and repeatedly extracted with ether (12 to 15 extractions). The extract, washed and dried, yielded only a small quantity of gummy material from which nothing definite could be obtained.

Isolation of an Acid

After further evaporation under reduced pressure to a volume of about 800 cc. the aqueous solution was heated to boiling and repeatedly shaken with hot amyl alcohol. The amyl alcohol was removed from the extract by distillation *in vacuo* and, finally, after addition of a little water, by steam distillation. Water was removed under reduced pressure from the residual solution, and the resulting syrup dissolved in ethyl acetate, the filtered solution being poured into ten times its volume of ether. This precipitated a gummy substance from which the supernatant liquor was decanted and evaporated to a small volume. The latter on standing, deposited a small quantity of acicular crystals which were recrystallized from benzene containing a little methanol, m.p. 183-184° C. From a solution of the precipitate in methanol (charcoal), a further crop of the same crystalline substance was obtained. Calcd. for $C_5H_8O_5$: C, 40.54; H, 5.45%; M.W. 148. Found: C, 40.97, 40.67; H, 5.31, 5.27%; M.W. 145, 147 (Rast). The product is acidic and is removed from its amyl alcoholic solution by aqueous potassium carbonate. A review of the literature failed to reveal any record of a substance of this composition, having identical properties.

To the aqueous solution, which had been extracted with amyl alcohol and freed of this solvent by distillation in a current of steam, a hot solution of basic lead acetate was added as long as a precipitate formed. This was filtered and washed by decantation with hot water. The combined filtrate and washings were deleaded with hydrogen sulphide and evaporated under diminished pressure to a syrup. Methanol was then added and the filtered solution again evaporated, the operation being repeated several times to remove the inorganic material. A small portion of the final residue, dissolved in water, and treated with phenylhydrazine yielded phenylglucosazone, m.p. 201° C. The main portion of the residue, dissolved in methanol and allowed to stand for several days, failed to yield anything crystalline. The solution was then evaporated and the residue boiled for five minutes in 5% aqueous sodium hydroxide, cooled rapidly and extracted with ether. This ethereal solution was shaken with 5% aqueous solutions of ammonium carbonate, potassium carbonate and sodium hydroxide, but failed to yield anything crystalline, and was discarded.

Isolation of i-Inositol, $C_6H_6(OH)_6$

The basic lead acetate precipitate was suspended in water and decomposed with hydrogen sulphide led into the vigorously stirred suspension. The precipitated lead sulphide was filtered, the filtrate distilled under reduced pressure to a thick syrup which was dissolved in methanol, and the solution allowed to stand at room temperature for several days. Small, transparent, prismatic needles were gradually deposited which, after several recrystallizations (charcoal) from methanol, melted at 223-224° C. This crystalline substance is very soluble in water, soluble in alcohol, but insoluble in ether. The salmon color produced by inositol in Scherer's test (6) was obtained with this substance. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.67%. Found: C, 40.11; 40.08; H, 6.83, 6.75%. Admixed with a known sample of *i*-inositol, the melting point was unchanged. The presence of *i*-inositol in the plant extract is, therefore, established.

Isolation of an Unidentified Substance

The methanolic mother liquor from the crystallization of *i*-inositol, when allowed to evaporate further, spontaneously deposited transparent, flat, elongated plates which, recrystallized from methanol or water, softened at 105° C. and melted completely at 120° C. The product is inert towards Fehling's solution but when reduced with sodium amalgam at 0° C. in a solution kept slightly acid with sulphuric acid, its power for reducing Fehling's solution gradually increases as the reaction proceeds. *d*-Allonolactone, prepared by Levene and Jacobs (5), has a similar percentage composition, identical melting point characteristics and behaves similarly towards Fehling's solution, but a sample kindly supplied by Dr. Levene depresses the melting point when admixed with it. *l*-Allonolactone recently prepared by Professor W. C. Austin (1), and kindly supplied by him, also proved to be different. Calcd. for $C_6H_{10}O_6$: C, 40.4; H, 5.62%; M.W. 178; calcd. for $C_7H_{12}O_7$: C, 40.39; H, 5.77%; M.W. 208. Found: C, 41.02, 41.01; H, 6.02, 6.02%; M.W. 196, 184 (Rast). The substance does not react with phenylhydrazine. Owing to the small quantity available, a more thorough study will necessarily await the collection of a new lot of material.

Examination of the Resinous Material (B)

The soft, resinous material filtered from the water-insoluble extract was mixed with shredded asbestos with the aid of alcohol which was subsequently evaporated. The hard cake thus formed was ground and extracted in a Soxhlet apparatus successively with petroleum ether, ether, chloroform, ethyl acetate and methanol.

Petroleum Ether Extract

After removal of the solvent by distillation, the residual oil was mixed with the petroleum ether extract of the original material, and the combined oil saponified with alcoholic potassium hydroxide. The cooled basic solution was diluted with an equal volume of water and shaken repeatedly with ether.

Isolation of Myricyl Alcohol, $C_{30}H_{62}O$

The solvent was distilled from the ether extract, the crystalline residue dissolved in boiling acetone and the filtered solution allowed to cool. The crystalline deposit, filtered and washed with cold acetone, was recrystallized several times from hot acetone, and distilled *in vacuo;* it came over at 300° C./2 mm. Recrystallized from ethyl acetate, it melted at 82-83° C. Calcd. for $C_{30}H_{62}O$: C, 82.19; H, 14.18%. Found: C, 82.28, 82.25; H, 14.02, 14.02%. This substance was thus identified as myricyl alcohol.

Isolation of Arisaesterol

After separation of myricyl alcohol, the acetone was evaporated from the mother liquor, and the crystalline residue dissolved in boiling methanol from which the sterol separated as narrow, lustrous plates. Recrystallized several times from hot methanol, the latter melted at 135° C. Calcd. for $C_{36}H_{64}O_2$: C, 81.82; H, 12.12%. Found: C, 81.79; H, 12.17%. This sterol, therefore, belongs to a class of the general formula $C_nH_{2n-8}O_2$. It is proposed to designate it as arisaesterol.

Isolation of Fatty Acids

The fatty acids obtained from the basic solution which has been extracted with ether were separated by the lead-soap–alcohol method into unsaturated and saturated fractions. The unsaturated acids were oxidized with cold, dilute permanganate and the product, by repeated extraction with boiling ethyl acetate, separated into tetrahydroxystearic acid, m.p. 172-173° C., and a small quantity of dihydroxystearic acid, m.p. 126° C. Admixture of the respective acids with authentic specimens caused no depression in melting point. Hence, the unsaturated acids appear to consist of a mixture of linoleic acid with a small amount of oleic acid.

The saturated acids were converted into the methyl esters and fractionated under diminished pressure. The first fraction, 150-156° C./3.5 mm., forming about one-fifth of the total, solidified on cooling to stellate aggregates of stiff, white needles, m.p. 28° C., and consisted of methyl palmitate. At 160-190° C./3.5 mm. a very small amount of a colorless oil distilled over, which, on standing, deposited acicular crystals of methyl palmitate. The residue in the flask was rinsed out with boiling methanol (charcoal) and the clear solution, on cooling, deposited fine, elongated flakes, m.p. 55-56° C. On hydrolysis, the acid was obtained in too small a quantity to permit definite identification.

Ether Extract (Isolation of a Phytosterolin)

The ethereal solution when concentrated to a convenient bulk, deposited a crystalline substance which, when recrystallized from a mixture of pyridine and alcohol, melted at 297° C. Calcd. for $C_{33}H_{56}O_6$: C, 72.25; H, 10.21%. Found: C, 71.76, 71.88; H, 10.49, 10.46%. There is little doubt that this is a phytosterol glucoside belonging to the series $C_nH_{2n-10}O_6$; its behavior in the sterol color reactions, and in Whitby's test for sterolins (8), together with

the failure to cause any depression in melting point when admixed with a specimen of a sterolin obtained from another plant, support this conclusion.

Chloroform, Ethyl Acetate and Methanol Extracts

From the chloroform solution a small quantity of a crystalline substance, m.p. 272° C. (decomp.) was isolated, but this was too minute to permit further work. The ethyl acetate and methanolic solutions yielded gums from which, even after boiling with 10% aqueous sodium hydroxide, nothing crystalline could be obtained.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLVI. STRUCTURE OF THE CELLULOSE SYNTHESIZED BY THE ACTION OF *ACETOBACTER XYLINUS* ON FRUCTOSE AND GLYCEROL[1]

By J. Barsha[2] and Harold Hibbert[3]

Abstract

The membranes synthesized by the action of *Acetobacter xylinus* on fructose and on glycerol have been shown (after suitable purification) by the recognized methods of methylation, acetylation, acetolysis and hydrolysis to be chemically identical with cotton cellulose. These conclusions are confirmed by the results of X-ray analysis.

The X-ray investigation by W. A. Sisson and G. L. Clark indicates the identity of the cellulose membranes formed from glucose, fructose, glycerol, sucrose, galactose and mannitol by the action of *Acetobacter xylinus* and thus lends support to the view that the same polysaccharide is synthesized by the organism whenever cell-wall formation (growth) occurs and that this polysaccharide is chemically identical with cotton cellulose.

Introduction

The formation of cellulose in plants has been studied by relatively few investigators, and it has been generally assumed, in the absence of experimental data, that glucose is the starting material from which cellulose is synthesized.

The knowledge that a simple unicellular organism, *Acetobacter xylinus*, is capable of producing cellulose in the formation and growth of its cell-wall (2, 3) indicated the possibility of using this organism for a study of the substances capable of acting as a starting material for cellulose formation. The fact that the membrane produced during the growth of the bacteria has, after purification, an empirical composition corresponding to $C_6H_{10}O_5$ and gives the so-called "cellulose reaction," namely a violet coloration with zinc chloride and iodine, was regarded by earlier workers (2, 3, 4) as sufficient proof of the cellulosic nature of the membrane. However, Lüdtke (13, 14) has shown that the color reaction is also given by other cell-wall polysaccharides, namely Mannan B and Xylan B. Pringsheim (16, p. 104) believes that the iodine-zinc chloride reaction is not a sufficient differentiation between cellulose and the hemicelluloses. Futhermore, Jordon states, "Among the bacteria, as in the lower fungi generally, cellulose is conspicuous by its absence, but another and somewhat similar carbohydrate, designated as hemicellulose, is often present in abundance" (11, p. 75).

It was therefore necessary, in studying the growth of *Acetobacter xylinus* and the accompanying formation of the cell-wall polysaccharide, to show, in several representative cases, that the polysaccharide of the purified cell-wall substance is actually cellulose.

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The selection of the polysaccharide product obtained during growth of the organism on glucose seemed to be the logical first choice. Complete chemical examination showed this polysaccharide to possess the same chemical structure as cotton cellulose (7). The present investigation relates to the constitution of the cell-wall substance represented by the membrane produced by the action of *Acetobacter xylinus* on fructose and on glycerol. Also in these cases it has been possible to show that the membranes, after suitable purification, have the same empirical composition, *viz.*, $C_6H_{10}O_5$.

Acetylation of the two bacterial celluloses with a mixture of acetic anhydride, glacial acetic acid and sulphuryl chloride as catalyst gave good yields of cellulose triacetates having a specific rotation in chloroform very similar to that of cellulose triacetate prepared from cotton cellulose. Saponification of the triacetates with $2N$ methyl alcoholic sodium hydroxide yielded regenerated celluloses which were shown by analysis to have the same empirical composition as the original bacterial celluloses ($C_6H_{10}O_5$).

Hydrolysis of the cellulose triacetates with methyl alcohol containing 0.9% of hydrogen chloride gave good yields of α- and β-methylglucosides. Direct hydrolysis of the regenerated celluloses (chosen in preference to the original cellulose because of their more finely divided form) with a hydrochloric acid solution of zinc chloride was followed polarimetrically and it was found that the end specific rotation approximated very closely to that of pure glucose in the same solvent.

Further proof of the chemical identity of the two bacterial celluloses was found in the experimental proof of the same type of linkage (1, 4) between the glucose units. Trimethyl celluloses were prepared by deacetylation and methylation of acetone-soluble cellulose acetates, followed by further methylations. The respective trimethyl celluloses yielded, on hydrolysis, 2,3,6-trimethyl methylglucoside which was hydrolyzed further to 2,3,6-trimethyl glucose. Additional confirmation was obtained in the preparation of cellobiose octacetate by direct acetolysis of the bacterial celluloses with acetic anhydride and concentrated sulphuric acid.

Unlike the quantitative conversion of starch to maltose, the decomposition of cellulose to cellobiose octacetate has never resulted in high yields, owing presumably to the further decomposition of this latter substance to glucose pentacetate. The best yields reported in the literature, *e.g.* Spencer* (18), 45%, were obtained during extensive investigations on the determination of the optimum conditions for acetolysis of cotton cellulose.

It was not deemed advisable in the present investigation to determine the optimum conditions for cellobiose octacetate formation from the bacterial celluloses because of the length of time required.

In an investigation of the α-celluloses from five different kinds of wood, Bell (1) found that these substances apparently contained a cellulose fraction more resistant to complete methylation so that the maximum methoxyl content attained was only 36.3–39.1%. Direct methylation of bacterial cellulose (prepared from glucose) with dimethyl sulphate and 30% sodium

hydroxide yielded a product with a methoxyl content of 43.9%, indicating the absence in the bacterial cellulose of a similar "resistant portion" such as was found by Bell in the wood α-celluloses.

On the basis of the experimental results obtained in this and other investigations (7) it would seem that the same polysaccharide is synthesized by *Acetobacter xylinus* whenever cell-wall formation (growth) occurs and that this polysaccharide is chemically identical with cotton cellulose.

Further support is given to the above conclusion in the results of X-ray examination of a number of bacterial celluloses prepared in this laboratory. The X-ray work was carried out by Dr. W. A. Sisson in collaboration with Dr. George L. Clark, Department of Chemistry, University of Illinois. They found (17) the purified bacterial membranes to be composed of well developed crystallites of cellulose which have a preferential orientation with respect to the surface of the sheet, similar to that reported by Mark and Susich (15) for stretched tunicin and "biosynthetic cellulose." In the case of the membranes prepared from mannitol and galactose, the specimens of the wet and highly swollen membranes were dried under a greater tension than the other samples, with the result that they show a greater degree of preferred orientation (as shown by the intensity being greater over certain sections of the X-ray diffraction rings).

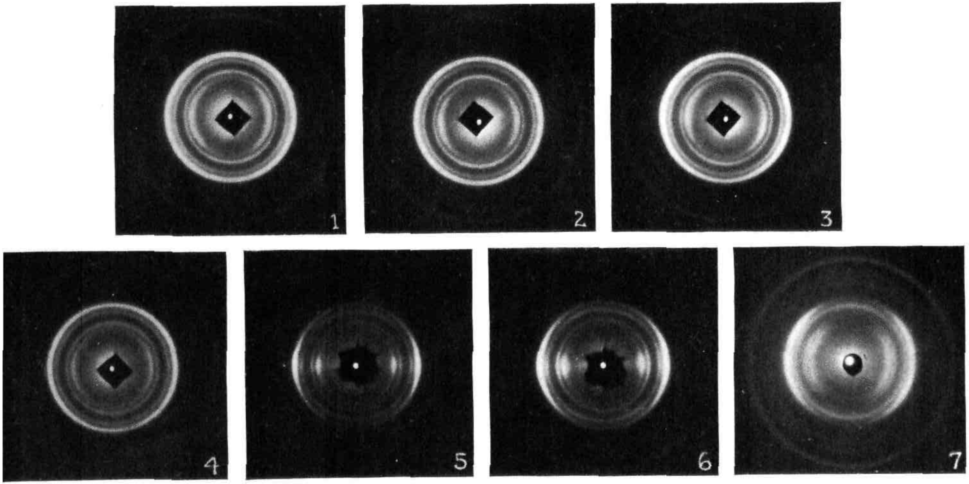
The synthesis of the same polysaccharide, cellulose, by *Acetobacter xylinus* from glucose, fructose, glycerol, sucrose, mannitol and galactose, would indicate the ability of the organism to carry out the many transformations required to convert all of these substances into the common product which serves as the starting material for cellulose formation. It has been generally assumed (5, 12, 19), in the absence of positive experimental proof, that this starting material is glucose.

A comparison of the compounds prepared from cotton and bacterial celluloses is given in Table I.

Experimental

Preparation, Purification and Analysis of Bacterial Celluloses from Fructose and Glycerol

The detailed procedure for the preparation from fructose and glycerol of the bacterial celluloses used in this investigation has been described by Tarr and Hibbert (20). In brief, a solution containing 5–10% of the carbon compound (fructose or glycerol), 1% potassium dihydrogen phosphate, 0.2% sodium chloride and 0.2% asparagine was inoculated with *Acetobacter xylinus* and incubated for about two weeks at 30° C. Examination under the microscope of the highly swollen membrane which formed on the surface of the liquid in the culture vessel revealed, after staining, a conglomeration of rod-like bacterial cells. It is to be noted that in the preparation of the membranes, a nutrient medium was used which did not support growth in the absence of the carbon compound.



X-ray diagrams of cotton and bacterial celluloses. FIGS. 1-6. *Bacterial cellulose from: 1, glucose; 2, fructose; 3, glycerol; 4, sucrose; 5, galactose; 6, mannitol.* FIG. 7. *Cotton fibre.*

TABLE I

COMPARISON OF COMPOUNDS PREPARED FROM COTTON AND BACTERIAL CELLULOSES

Starting material	Cellulose triacetate		α- and β-methyl glucosides from cellulose triacetate		Trimethyl cellulose			
	*$[\alpha]_D$ in $CHCl_3$	Yield, %	Equil. *rotation in* CH_3OH–HCl	Yield, %	$[\alpha]_D$ in $CHCl_3$	M.p.,† ° C.	OCH_3, %	Yield, %
Cotton cellulose (6, 9, 10, 18)	−22.3°	99.5	+106–108°	95.5	−10°	215–216	45.6	85–98
Bacterial cellulose from:								
glucose (7)	−21.9°	98.8	+107.5°	94.1	− 9.2°	231–232	44.0	84.6
fructose	−21.0°	94.5	+105.9°	92.5	− 7.9°	227–230	44.1	82.6
glycerol	−21.8°	97.9	+105.3°	92.2	− 8.8°	232–234	44.4	79.4

Starting material	2,3,6-Trimethyl methylglucoside				2,3,6-Trimethyl glucose			Cellobiose octacetate		
	$[\alpha]_D$ in $CHCl_3$	B.p., ° C.	Refractive index	Yield (crude), %	$[\alpha]_D$ in CH_3OH	M.p., ° C.	Yield, %	$[\alpha]_D$ in $CHCl_3$	M.p., ° C.	Yield, %
Cotton cellulose (6, 9, 10, 18)	+66.0°	115–118 (0.5 mm.)	1.4590	95	+66.5°	104–108	86	41–41.8°	227.5–228	45
Bacterial cellulose from:										
glucose (7)	+64.4°	111.5–115 (0.35–0.40 mm.)	1.4560 (25° C.)	92.3	+65.2°	105–108	83.5	41.0°	222–223	27.3
fructose	+66.7°	110–113 (0.25–0.30 mm.)	1.4555 (25° C.)	96.0	+66.2°	104–106	96.8	40.8°	222–223	29.0
glycerol	+68.5°	109–113 (0.25 mm.)	1.4553 (25° C.)	99.0	+67.7°	102–104	87.0	40.4°	223.5–224	22.9

* *For temperatures see Experimental Part.*
† *The difference in melting point is due to the incompleteness of the methylation.*

The membranes were washed with repeated changes of distilled water to remove the water-soluble materials, including the sugars, until the wash waters no longer reduced Fehling's solution. They were then immersed in 2% sodium hydroxide solution, placed in an autoclave (100° C.) for 30 min. and then washed with distilled water until neutral to phenolphthalein. To remove last traces of alkali they were immersed in cold 1% acetic acid and finally washed with large volumes of distilled water. Microscopic examination of the moist membrane after washing showed that the bacterial cells had been disintegrated since no structural forms were visible.

The yield of the pure dried membrane was relatively low, *i.e.*, 2–5% of the weight of the carbon compound used as substrate. The cellulose so obtained from fructose and from glycerol* is completely insoluble in all ordinary organic solvents. The usual fusion test with potassium showed the absence of nitrogen. The combustion analyses were carried out on samples which had been dried at 100° C./1 mm. over phosphorus pentoxide. Analysis:—*A*, Found: C, 44.81, 44.20; H, 6.38, 6.36%. Calcd. for $C_6H_{10}O_5$: C, 44.42; H, 6.20%. *B*, Found: C, 44.58, 44.32; H, 6.31, 6.36%.

Preparation of Cellulose Triacetate

This was carried out according to the modification of Barnett's method devised by Irvine and Hirst (9). One part of bacterial cellulose was acetylated at 65–70° C. with 7.5 parts of glacial acetic acid and 17.5 parts of acetic anhydride with nascent sulphuryl chloride as catalyst. The acetylation mixture was diluted with two volumes of acetone, dropped into five volumes of distilled water at 90° C. whilst stirring rapidly, and the precipitate filtered and washed thoroughly with distilled water until free from acid.

Bacterial cellulose *A* (10 gm.; moisture, 6.51%) yielded 15.70 gm. of cellulose triacetate (94.5%). In chloroform, $[\alpha]_D^{25} = -21.0°$ (c = 1.666). Analysis:—Found: CH_3CO, 44.7%. Calcd. for $C_6H_7O_5(CH_3CO)_3$: CH_3CO, 44.8%.

Cellulose *B* (9.47 gm.; moisture, 6.22%) yielded 15.45 gm. of cellulose triacetate (97.9%). In chloroform, $[\alpha]_D^{27} = -21.8°$ (c = 1.231). Analysis:—Found: CH_3CO, 44.2%.

Regeneration of Cellulose from the Cellulose Triacetates A and B

Two grams of each cellulose triacetate was saponified by immersing in 50 cc. of 2*N* methyl alcoholic sodium hydroxide solution at room temperature for 24 hr. The regenerated cellulose was filtered and washed thoroughly until free from alkali.

Cellulose triacetate *A* (2.00 gm.) yielded 1.10 gm. of regenerated cellulose (97.8%). Analysis:—Found: C, 43.98; H, 6.23%. Calcd. for $C_6H_{10}O_5$: C, 44.42; H, 6.20%.

Cellulose triacetate *B* (2.00 gm.) yielded 1.03 gm. of regenerated cellulose (91.6%). Analysis:—Found: C, 44.23; H, 6.39%.

**Throughout the experimental part of this paper preparations derived from the bacterial cellulose prepared from fructose will be designated by A whilst those derived from the bacterial cellulose prepared from glycerol will be designated by B.*

Hydrolysis of the Cellulose Triacetates A and B to α- and β-Methylglucosides

About four grams of each cellulose triacetate was hydrolyzed with 60 cc. of dry methyl alcohol containing 0.9% hydrogen chloride in a sealed bomb-tube at 125° C. for 60 hr. The acid was then neutralized with silver carbonate, the solution filtered and the filtrate decolorized with charcoal. The alcohol was evaporated under diminished pressure in a tared flask. The semi-crystalline product was redissolved in hot ethyl alcohol which was then evaporated at 60° C./10 mm. A crystalline product was obtained.

Dry cellulose triacetate *A* (3.82 gm.) yielded 2.37 gm. of α- and β-methylglucosides (92.5%). In a solution of methyl alcohol containing 0.9% hydrogen chloride, the mixed glucosides showed $[\alpha]_D^{26} = +97.8°$ (c = 0.9924). After heating in a sealed tube at 100° C. for six hours a portion of this same solution had a constant specific rotation of +105.9°. Fractional crystallization of the mixed glucosides from absolute ethyl alcohol yielded the characteristic crystals of α-methylglucoside melting at 163.5° C. In water, $[\alpha]_D^{26} = +156.6°$ (c = 0.920). The mother liquor from the recrystallization, which contained an excess of β-methylglucoside, was evaporated to dryness under diminished pressure and the residue was dissolved in methyl alcohol containing 0.9% hydrogen chloride. This solution was heated for 15 hr. in a sealed tube at 100° C. to re-establish the equilibrium. A portion of this solution showed $[\alpha]_D^{26} = +106.3°$ (c = 1.016).

Dry cellulose triacetate *B* (3.96 gm.) yielded 2.46 gm. of α- and β-methylglucosides (92.2%). A solution of the mixed glucosides in methyl alcohol (0.9% hydrogen chloride) showed $[\alpha]_D^{26} = +96.5°$ (c = 1.026) which increased on heating as above to a constant specific rotation of +105.3°. Fractional crystallization yielded α-methylglucoside; m.p. 165° C. In water, $[\alpha]_D^{25} = +156.7°$ (c = 0.5934). Re-establishment of the equilibrium was brought about by heating as before. In methyl alcohol (0.9% hydrogen chloride), $[\alpha]_D^{26} = +105.2°$ (c = 1.112).

Hydrolysis of Cellulose A and Cellulose B with Zinc Chloride-Hydrochloric Acid

The method employed was that of Hibbert and Percival (8) in which the course of the hydrolysis of cellulose dissolved in zinc chloride-hydrochloric acid is followed by observing the change in rotation of the solution. The procedure has been described in detail elsewhere (7, p. 587). In the same solvent, pure anhydrous glucose showed $[\alpha]_D^{23} = +67.5°$ (c = 1.0028).

Regenerated cellulose *A* (0.5049 gm.) was dissolved in 50 cc. of solvent (c = 1.0098). The observed specific rotations are given in Table II and plotted in Fig. 8.

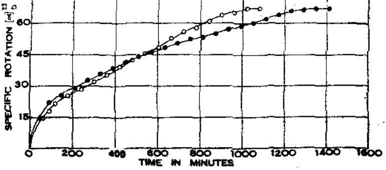


FIG 8. *Hydrolysis curves:* •, *bacterial cellulose A (from fructose);* o, *bacterial cellulose B (from glycerol).*

TABLE II

HYDROLYSIS OF BACTERIAL CELLULOSE *A* IN ZINC CHLORIDE–HYDROCHLORIC ACID SOLUTION

Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$
0	0	390	38.8	810	53.3	1230	65.7
45	+14.6°	450	41.6	870	55.0	1290	66.3
90	22.0	510	44.0	930	57.0	1350	66.8
150	25.5	570	46.2	990	58.4	1410	+66.8
210	29.1	630	48.4	1050	60.1		
270	32.8	690	50.4	1110	62.0		
330	36.0	750	52.2	1170	64.0		

Regenerated cellulose *B* (0.2536 gm.) was dissolved in 50 cc. of solvent (c = 0.5072). The observed specific rotations are given in Table III and plotted in Fig. 8.

TABLE III

HYDROLYSIS OF BACTERIAL CELLULOSE *B* IN ZINC CHLORIDE–HYDROCHLORIC ACID SOLUTION

Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$	Time, min.	$[\alpha]^{23}_D$
0	0	240	28.3	540	45.5	840	60.9
60	+14.8°	300	32.0	600	48.6	900	64.0
90	17.9	360	35.1	660	52.9	960	65.2
120	21.5	420	39.4	720	56.0	1020	67.1
180	25.2	480	42.5	780	57.8	1080	+67.1

Preparation of Trimethyl Cellulose by Deacetylation and Methylation of Cellulose Acetates A and B

Cellulose acetate *A* (10.00 gm.; CH_3CO, 42.3%) was dissolved in 300 cc. acetone and simultaneous deacetylation and methylation effected with 320 cc. of sodium hydroxide solution (30% by weight) and 120 cc. of dimethyl sulphate. The reagents were added in 10 portions, one every 15 min. at 56° C. At the end of the reaction, the acetone was distilled off, the mixture heated to 95° C., filtered immediately through a steam jacketed Büchner funnel and washed thoroughly with boiling distilled water. Analysis:—Found: CH_3O, 38.13%. Calcd. for trimethyl cellulose: CH_3O, 45.6%.

The product was dissolved in 125 cc. of pyridine and reacetylated with 70 cc. of acetic anhydride at 50° C. for five days. The solvents were removed at 40–50° C./10 mm. The residue was taken up in 500 cc. of benzene and precipitated by dropping into 1250 cc. of cold petroleum ether (b.p. 30–50° C.) with rapid stirring. It was centrifuged and washed with more petroleum ether. This material was then suspended in 150 cc. of acetone and methylated as before. Analysis:—Found: CH_3O, 42.74%. The product was subjected to reacetylation and a third methylation. Analysis:—Found: CH_3O, 43.33%.

A final methylation was then performed with Purdie's reagents as described previously (7, p. 589). Yield from cellulose acetate *A*; 6.00 gm. (82.6%

of theory for trimethyl cellulose). Analysis:—Found: C, 52.86, 52.27; H, 7.88, 7.77; CH_3O, 44.1%. Calcd. for $C_6H_7O_5(CH_3)_3$: C, 52.91; H, 7.90; CH_3O, 45.6%. The trimethyl cellulose *A* showed in chloroform, $[\alpha]_D^{26} = -7.9°$ (c = 1.262); in benzene $[\alpha]_D^{26} = -14.2°$ (c = 1.057). M.p., 227–230° C.

Cellulose acetate *B* (12.00 gm.; CH_3CO, 41.0%) was dissolved in 240 cc. of acetone and methylated in the same manner with 385 cc. of 30% sodium hydroxide solution and 145 cc. of dimethyl sulphate. Yield, 8.32 gm. (93.4%). Analysis:—Found: CH_3O, 41.7%.

A portion (4.0 gm.) of the product was dissolved in 40 cc. of pyridine and acetylated with 10 cc. of acetic anhydride for 48 hr. at room temperature. The product, obtained as before, was suspended in 100 cc. of acetone and methylated in the usual way. Analysis:—Found: CH_3O, 44.0%. A second reacetylation and methylation produced no increase in the methoxyl content.

Part of the methylated product (3.5 gm.) was dissolved in 110 gm. of methyl iodide and treated as before with 40 gm. of dry silver oxide. Yield, 3.4 gm. (79.4%). Analysis:—Found: C, 53.05, 52.65; H, 8.10, 8.00; CH_3O, 44.4%. Trimethyl cellulose *B* showed, in chloroform, $[\alpha]_D^{25} = -8.8°$ (c = 1.130); in benzene, $[\alpha]_D^{26} = -15.2°$ (c = 1.055). M.p., 232–234° C.

Direct Methylation of Bacterial Cellulose (from Glucose)

Dry bacterial cellulose (5.13 gm., prepared by the action of *Acetobacter xylinus* on glucose) was suspended in 350 cc. of acetone and methylated with 200 cc. of dimethyl sulphate and 460 cc. of sodium hydroxide solution (30%) at 56° C. The product was worked up in the usual manner. Eight such methylations in all were carried out. Yield, 5.20 gm. (80.5%). Analysis:—Found: CH_3O, 43.93%.

Hydrolysis of Trimethyl Celluloses A and B to 2,3,6-Trimethyl Methylglucosides

Well dried trimethyl cellulose *A* (2.70 gm.; CH_3O, 44.1%) and 40 cc. of pure methyl alcohol containing 1% of hydrogen chloride were heated in a bomb tube at 100° C. for 50 hr. as already described (7, p. 589). Yield, 3.00 gm. (96% of theory, calculated for conversion of trimethyl cellulose to trimethyl methylglucoside).

The product was dissolved in dry ether and transferred to a 5-cc. Claissen flask in which the ether was evaporated off. The trimethyl methylglucoside was then distilled under diminished pressure. A colorless, viscous liquid (2.64 gm.) was obtained; b.p., 110–113° C./0.25–0.30 mm. Refractive index = 1.4555 (25° C.). In chloroform, $[\alpha]_D^{24} = +66.9°$ (c = 1.151). Analysis:—Found: CH_3O, 52.3%. Calcd. for $C_6H_8O_6(CH_3)_4$: CH_3O, 52.6%.

Trimethyl cellulose *B* (2.13 gm.; CH_3O, 44.4%) and 35 cc. of pure methyl alcohol containing 1% of hydrogen chloride were heated in a bomb tube at 100° C. for 50 hr. The product was isolated as before. Yield, 2.44 gm. (99.0%). Distillation yielded 2.13 gm. of a colorless, viscous liquid. B.p., 109–113° C./0.25 mm. Refractive index, 1.4552 (25° C.). In chloroform, $[\alpha]_D^{26} = +68.5°$ (c = 1.358). Analysis:—Found: CH_3O, 51.1%.

Preparation of 2,3,6-Trimethyl Glucose from Trimethyl Celluloses A and B

Trimethyl methylglucoside *A* (2.02 gm.) dissolved in 125 cc. of 5% hydrochloric acid was heated under reflux for 10 hr. in the manner described (7, p. 590). A sirup was obtained which crystallized on cooling. Yield, 1.84 gm. (96.8%). After one recrystallization from dry ether, the needle-like crystals melted at 104–106° C. In methyl alcohol, containing a trace of hydrogen chloride, the product showed $[\alpha]_D^{26} = +66.2°$ (c = 0.5287).

Trimethyl methylglucoside *B* (1.65 gm.) was hydrolyzed under reflux with 100 cc. of 5% hydrochloric acid solution for 12 hr. The product was worked up as before. Yield, 1.35 gm. (87%). One recrystallization from dry ether yielded crystals melting at 102–104° C. In methyl alcohol containing a trace of hydrogen chloride, $[\alpha]_D^{25} = +67.7°$ constant (c = 0.992).

Acetolysis—Preparation of Cellobiose Octacetates A and B

Dry bacterial cellulose *A* (2.03 gm.) was subjected to acetolysis by treatment with 8 cc. of acetic anhydride and 0.2 cc. of concentrated sulphuric acid for 19 days at 50° C. The reaction mixture was then diluted with 10 cc. of glacial acetic acid, well stirred, and dropped into 500 cc. of cold distilled water. After standing overnight at room temperature, the precipitate was filtered and washed with distilled water until free from acid. The air dried precipitate was then extracted under reflux with three 100-cc. portions of 95% alcohol and filtered hot. The combined extracts were decolorized with charcoal and filtered hot. The crystals of cellobiose octacetate which separated out on cooling were filtered on to a tared Gooch crucible. The mother liquor was evaporated to 50 cc. and cooled. A further amount of cellobiose octacetate was obtained. Yield, 1.23 gm. (29%). After two recrystallizations from ethyl alcohol, the cellobiose octacetate showed, in chloroform, $[\alpha]_D^{24} = +40.8°$ (c = 1.777) and melted at 222–223° C.

Bacterial cellulose *B* (2.10 gm.) was subjected to acetolysis as above. Yield, 1.05 gm. (22.9%). (A small amount of product was accidentally lost during extraction.) After two recrystallizations from ethyl alcohol, the cellobiose octacetate showed, in chloroform, $[\alpha]_D^{23} = +40.4°$ (c = 0.790). M.p., 223.5–224° C.

Bacterial cellulose (1.96 gm., prepared from glucose) was subjected to acetolysis. Yield, 1.12 gm. of cellobiose octacetate (27.3%). The product was recrystallized twice from ethyl alcohol. In chloroform, $[\alpha]_D^{22} = +41.0°$ (c = 0.712). M.p., 222–223° C.

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THE INFLUENCE OF TEMPERATURE ON THE RATE OF NATURAL PENETRATION OF ELECTROLYTES INTO WOOD[1]

BY O. MAASS[2]

Abstract

The rates of diffusion of sodium hydroxide, sodium chloride and hydrochloric acid into chips of black spruce heartwood impregnated with water were measured for different shapes of chip at 20, 50, and 75° C. In the longitudinal direction of the wood, hydrochloric acid diffuses the most rapidly and sodium hydroxide and sodium chloride at nearly the same rates. In the lateral direction sodium hydroxide diffuses the most rapidly, owing to its action on the wood. Concentration of electrolyte is practically without influence on the time to half-value for sodium hydroxide and hydrochloric acid. The density of the wood does not affect the rate of diffusion of sodium hydroxide.

The rate of diffusion of water into air dry chips was measured at 20 and 50° C.

Introduction

In order to understand the mechanism of the cooking of wood with alkalies, it is necessary to understand related phenomena. Among these are included: *(a)* the sorption of sodium hydroxide on wood; *(b)* the manner in which liquids penetrate into wood containing air, and the influence of various factors on the rate at which impregnation of the wood takes place; *(c)* the natural penetration, or diffusion, of the cooking reagent into chips during the cooking process when the chips are completely impregnated with liquor.

Data relating to these three problems were obtained by Richardson and Maass (2), who carried out experiments at room temperatures. The present work was begun as an investigation of the influence of temperature on the rate of diffusion of electrolytes into wood, with a view to ascertaining the relation of data to actual cooking conditions. At the same time some experiments were made on the rate of natural penetration of water into air dry wood. Experiments have also been carried out in an attempt to explain the differences in the rates of diffusion of sodium hydroxide, sodium chloride and hydrochloric acid into wood chips impregnated with water.

For comparison of results, the general details of experimental procedure as devised by Richardson and Maass (2) were followed in the present work; *i.e.*, chips of black spruce heartwood, of square cross section and weight 2.75 gm. (oven dry) were used. The concentration of the electrolytes was 1.27 molar except in the experiments on the effect of concentration. A modified form of diffusion cell, however, was used.

Fig. 1 shows a side-view of the diffusion cell, containing a chip surrounded by solution. *B* is a section of the conductivity cell by means of which the resistance of the solution was measured. The inner tube (2) was used with chips of length greater than 2.5 cm. The chief purpose of this inner tube was

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to minimize the amount of solution necessary to cover the chip completely, so that the concentration change would be as large as possible, with a resulting accuracy in measurement. The solution was drawn up into the inner tube and the level maintained by a clamp on a rubber tube attached to the inner tube at (4). When making a measurement this clamp was released, allowing the solution to run down into the main body of the cell where it was thoroughly stirred by alternately sucking up and blowing solutiou out of the electrode chambers and the inner tube. The temperature of the solution was kept constant throughout an experiment by placing the cell in a water bath equipped with an automatic temperature regulator and a constant level syphon to compensate for evaporation from the bath at the higher temperatures.

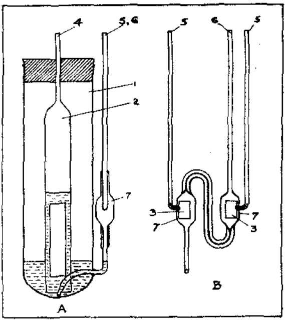

FIG. 1. *Diffusion cell.*

When a chip, impregnated with water, is placed in a solution of an electrolyte, the concentration changes as electrolyte diffuses into the chip. This change in concentration provides a means of measuring the rate of diffusion. Knowing the original concentration, the weight of solution and the weight of water in the chip, the final concentration can be calculated. The concentration corresponding to the diffusion of one-half of the electrolyte finally diffusing into the chip can then be calculated, which makes it unnecessary to continue an experiment much after the half-value has been reached. This procedure eliminates to a large extent errors caused by loss of water vapor from the cell at higher temperatures while waiting for the concentration to reach equilibrium.

The final and one-half values of the concentrations were changed to the corresponding resistance values, assuming the change in resistance to be proportional to the change in concentration, since the observed change in concentration was small. The relation between the changes in resistance and concentration was obtained in a control experiment, in which wood in the form of thin sections at right angles to the grain ("wood flake") was used instead of a chip. In such an experiment the diffusion was very rapid and the solution reached equilibrium before such errors as loss of water vapor became appreciable.

In the experiments with sodium hydroxide, the change in concentration was followed by titrating weighed samples of solution after known intervals of time, since it was found that the resistance did not reach a constant value. Besides the change in concentration due to the diffusion of electrolyte into the chip, there is a further change, amounting to about 25% of the total

change, due to sorption of alkali by the wood. The sorption value is known from experiment and its effect on the final concentration can be calculated. (In the cases of sodium chloride and hydrochloric acid sorption is negligible.)

One effect of sorption is to increase the amount of alkali which has to diffuse into a chip before the half-value is reached. At the same time, however, sorption increases the concentration gradient, which in turn would be expected to increase the rate of diffusion and so tend to offset the effect of the larger amount of alkali. Inasmuch as it is impossible to measure or to estimate the extent of these two effects, they have been neglected as far as the calculations are concerned. Because of sorption, the comparison of the rates of diffusion of sodium hydroxide with the values for sodium chloride and hydrochloric acid is not strictly valid. The measured rate of diffusion of sodium hydroxide is probably less than the true value, which would be obtained if sorption were absent.

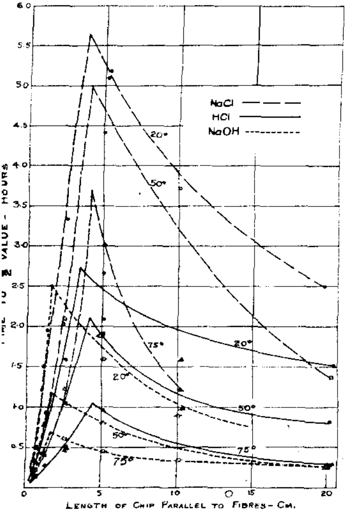

FIG. 2. *Diffusion of sodium chloride, sodium hydroxide, and hydrochloric acid into black spruce chips impregnated with water.*

Concentration in the case of sodium hydroxide, or resistance in the case of sodium chloride and hydrochloric acid, was plotted against time, and from the curve the time to half-value, or half-diffusion, was obtained. This quantity was used as a basis for comparison of rates of diffusion.

A consideration of the errors in measurement, and especially errors introduced by the use of temperatures as high as 75° C., showed that the accuracy obtained in these experiments was greater than that necessary, taking into account the variable nature of the wood.

Results and Discussion

Of those factors which influence the rate of diffusion the following have been investigated: (i) chip length, (ii) temperature change, (iii) concentration of electrolyte, and (iv) wood density.

Effect of (i) Chip Length, and (ii) Temperature Change

In Table I are given the data obtained for the three electrolytes at different temperatures and for different chip lengths. These values are plotted in Fig. 2.

TABLE I

EFFECT OF CHIP LENGTH AND TEMPERATE CHANGE

Sodium hydroxide		Hydrochloric acid		Sodium chloride	
Chip length, cm.	Time to half-diffusion, $T_{1/2}$, min.	Chip length, cm.	Time to half-diffusion, $T_{1/2}$, min.	Chip length, cm.	Time to half-diffusion, $T_{1/2}$, min.
At 20° C.					
.66	30	.17	3.0	.64	30
1.32	90	.66	19	1.30	116
2.59	125	1.22	47	2.57	200
4.88	113	2.57	95	5.24	310
5.13	95	5.10	125	5.16	305
10.3	53	5.08	158	19.8	150
		20.4	91		
At 50° C.					
.32	9.0	.18	2.0	.18	2.0
.69	23.0	.30	5.0	.38	6.5
1.29	46	.69	7.0	.69	16.0
2.54	63	1.22	25	1.30	55
5.16	47	2.54	93	2.36	121
10.3	20	5.03	115	5.03	264
20.0	16	10.1	73	10.08	222
		20.1	49	20.3	82
At 75° C.					
.64	13.0	.32	3.0	.32	6.0
1.22	25.0	1.30	16.0	1.27	25.0
2.59	36.0	2.54	27.5	2.54	73
5.11	26.0	5.08	57.0	5.08	180
10.4	20.0	20.3	17.0	10.2	58
20.3	16.0			10.1	95

It is seen that each curve gives a maximum value which corresponds to the length of chip into which the electrolyte concerned diffuses at a minimum rate. The other dimensions of this chip when calculated indicate the "worst shape" of chip. The manner in which this shape varies for the three electrolytes is illustrated in Fig. 3. The experimental accuracy, which is limited chiefly by the wood, does not appear to be such that a distinction may be made between the worst shapes of chip for sodium chloride and hydrochloric acid diffusions.

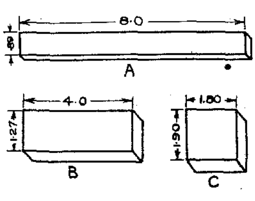

FIG. 3. *"Worst shape" of chips for diffusion of sodium hydroxide, sodium chloride, hydrochloric acid and water into black spruce. A, Water into air dry wood at 50° C.; B, sodium chloride and hydrochloric acid; C, sodium hydroxide.*

The rates of diffusion of electrolytes into chips of the worst shape decrease rapidly in the order sodium hydroxide, hydrochloric acid and sodium chloride (Fig. 4). Over the temperature range studied, the rate of diffusion increases in proportion to the temperature rise. The worst shape of chip for diffusion of sodium hydroxide is practically a cube, which means that the rate of diffusion per unit area of the side of a chip is about one-half that for the end of a chip. Under cooking conditions the alkali would be expected to open up the wood structure, so that diffusion in such a case is probably equally rapid into both side and end of a chip.

Furthermore, the rate of diffusion of sodium hydroxide into a chip in the form of a cube of about 1.8 cm. (0.7 in.) side increases about four times when the temperature is raised from 20 to 75° C. The indication then is that at cooking temperatures the rate of diffusion is so rapid into a chip of the much smaller size used in commercial practice that the concentration of alkali is practically constant in all parts of the chip being cooked. Chip shape appears to be of minor importance as far as the cooking operation is concerned, but inasmuch as the chips used commercially contain air, which is an almost unknown factor influencing the initial diffusion of liquor into the chips, the shape of chip should be such as to permit the most rapid impregnation of the chip with liquor.

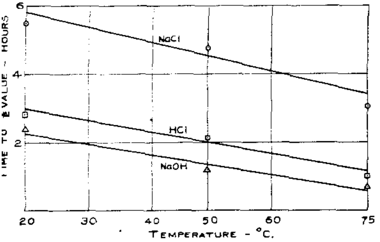

FIG. 4. *Diffusion of sodium hydroxide, hydrochloric acid and sodium chloride into chips of the "worst shape".*

(iii) Effect of Concentration of Electrolyte

Chips of length 0.64 cm. were used in these experiments, the results of which are given in Table II. It was concluded that concentrations above

TABLE II

EFFECT OF CONCENTRATION OF SODIUM HYDROXIDE

Molar conc. of NaOH	.24	.30	.50	1.06	2.9	6.6	13.5	13.5
$T_{½}$, min.	33.0	34.0	25.0	17.0	15.0	17.5	12.0	17.0

1.06 molar have no effect on the time to half-value for sodium hydroxide. Similar results were obtained with hydrochloric acid of concentrations between 0.38 and 1.37 molar (Table III). No experiments were made on the effect of concentration on the rate of diffusion of sodium chloride.

TABLE III

EFFECT OF CONCENTRATION OF HYDROCHLORIC ACID

Chip length, cm.	$T_{1/2}$, min.		Chip length, cm.	$T_{1/2}$, min.	
	0.38 *M* HCl	1.37 *M* HCl		0.38 *M* HCl	1.37 *M* HCl
0.64	7.0	8.0	1.27	25.0	21.0

(iv) Effect of Density of Wood

The wood used in these experiments was very close grained, having 40-60 annual rings per inch and a density of 0.55. The results are given in Table IV in comparison with values for wood having 15-20 rings per inch and a density of 0.40, and it is seen that the same time to half-value obtains in the case of both close grained and open grained wood. Wood density therefore does not influence the rate of diffusion of sodium hydroxide into a chip.

TABLE IV

EFFECT OF DENSITY OF WOOD ON RATE OF DIFFUSION OF SODIUM HYDROXIDE AT 20° C.

Chip length, cm.	$T_{1/2}$, min.		Chip length, cm.	$T_{1/2}$, min.	
	Close grained wood	Open grained wood		Close grained wood	Open grained wood
0.61	37	30	2.69	125	125
1.37	75	90	5.33	120	113

(v) Effect of Wood Structure

Information regarding the rates of diffusion of the three electrolytes in the two main directions of the wood structure may be obtained from a further study of the curves in Fig. 2. Theoretical considerations are also of interest in this connection, for the diffusion process is in a sense an ionic one, and, since hydrogen ions diffuse very rapidly, hydrochloric acid would be expected to diffuse the most rapidly of the three electrolytes studied, and sodium hydroxide and sodium chloride at nearly the same rates.

In a very short chip, most of the diffusion takes place through the ends of the chip, but in a very long chip most of the diffusion takes place through the sides. In the portion of an isotherm (Fig. 2) to the left of the maximum, then, longitudinal diffusion is the predominating effect, but for the right part of the curve lateral diffusion predominates. The left parts of the isotherms indicate that for longitudinal diffusion, hydrochloric acid diffuses the most rapidly, and sodium hydroxide and sodium chloride at nearly the same rates, as would be expected for reasons mentioned above. The right parts of the isotherms, however, indicate that while the expected order of the relative

rates of diffusion obtains in the cases of sodium chloride and hydrochloric acid, sodium hydroxide diffuses much more rapidly in the lateral direction of the wood structure than either of the other two electrolytes.

More data relating to the diffusion of sodium hydroxide and sodium chloride have been obtained in the following series of experiments in which were investigated: (1) the effect of pretreating a chip with sodium hydroxide, on the rate of diffusion of sodium chloride, (2) the rates of diffusion of sodium hydroxide and sodium chloride into chips whose sides were coated with wax so that diffusion could take place only in the longitudinal direction, and (3) the effect of concentration of the sodium hydroxide, used in pretreating the wood, on the rate of diffusion of sodium chloride. All these experiments were carried out at room temperature.

TABLE V

EFFECT OF PRETREATMENT WITH SODIUM HYDROXIDE ON RATE OF DIFFUSION OF SODIUM CHLORIDE INTO A CHIP (20° C.)

NaCl into untreated chip	NaCl into penetrated chip	NaOH into untreated chip
$T_{1/2}$, min.		
310	100	113
305	140	95

Sodium hydroxide was allowed to diffuse into a chip until sorption equilibrium was reached. The chip was then washed in water until all the alkali had diffused out. The rate of diffusion of sodium chloride into the pretreated chip was found to be almost as great as that of sodium hydroxide, as shown by the data in Table V, which were obtained in experiments on 5.1-cm. chips.

In order to find out whether the action of the alkali increases the longitudinal or the lateral diffusion of the sodium chloride, or both, a series of experiments was carried out on two 5.0-cm. presoaked chips whose sides were coated with wax. The rate of diffusion of sodium chloride was measured for each chip, which was then placed in water until all the sodium chloride had diffused out. The rate of diffusion of sodium chloride into the chip was measured again as check on the previous measurement and to see what accuracy might be expected in these experiments. Three more diffusion measurements were then made on each chip. The order in which the electrolytes were used was sodium hydroxide, sodium chloride and sodium hydroxide. One to two weeks was necessary between diffusion measurements in order to allow the electrolyte to diffuse completely out of the wood before another experiment could be made. The results of these experiments are given in Table VI.

In the first experiments with sodium chloride and sodium hydroxide, the latter appears to have diffused somewhat faster than the former, but experimental accuracy is not such that a definite conclusion may be drawn. The last diffusion experiments indicate that the previous treatment of the wood with sodium hydroxide decreased the rate of longitudinal diffusion for both electrolytes. This result may mean that there occurred swelling of the

membranes at the bordered pits, or of the walls of the tracheids, which reduced the effective diameter of the fibre cavities.

These experiments show that there is little difference in the rates of diffusion of sodium hydroxide and sodium chloride in the fibre direction of the wood. Since the rate of diffusion of sodium chloride into a chip of the shape used in these experiments is much less than that of sodium hydroxide, the lateral diffusion of sodium hydroxide must be much greater than that of sodium chloride, thus confirming a previous conclusion based on the curves of Fig. 2. The more rapid lateral diffusion of sodium hydroxide may be due to: (i) a swelling of the wood fibres in such a way that the wood structure is opened up, so that diffusion not only of sodium hydroxide but also of sodium chloride is greatly increased over the rate for sodium chloride into untreated chips; (ii) removal from the wood of resins, fats and other substances, which may be so distributed as to block passages through the cell walls which sodium hydroxide opens up by dissolving these substances.

TABLE VI

EFFECT ON RATE OF DIFFUSION OF TREATING CHIP WITH SEQUENCE OF DIFFERENT ELECTROLYTES

—	$T_{1/2}$, hr.	
	Chip No. 1	Chip No. 2
1st NaCl diffusion	24.5	23.0
2nd NaCl diffusion	22.0	24.5
1st NaOH diffusion	—	21.0
3rd NaCl diffusion	32.5	31.0
2nd NaOH diffusion	27.5	Over 30

If swelling is the explanation it would be expected that treating wood with sodium hydroxide of different concentrations should affect the increase in the rate of diffusion of sodium chloride to different extents. Six 2.5-cm. chips were impregnated with water and the rate of diffusion of sodium chloride into each chip was measured twice. Four of these chips were allowed to come to sorption equilibrium with sodium hydroxide of concentrations 1.4, 2.9, 6.2 and 10 molar respectively. After allowing the alkali to diffuse out of each chip, the rate of diffusion of sodium chloride was again measured twice. The fifth chip was placed in alcohol for several days and then in ether for five to six weeks. The chip was again placed in water and subjected to a vacuum to assist in removing the ether. The sixth chip was not used. The results of these experiments are given in Table VII.

TABLE VII

EFFECT OF PRETREATING WITH SODIUM HYDROXIDE OF VARIOUS CONCENTRATIONS ON RATE OF DIFFUSION OF SODIUM CHLORIDE INTO CHIPS AT 25° C.

Chip No.	$T_{1/2}$, min. (before treatment of chip)		Treating reagent	$T_{1/2}$, min. (after treatment of chip)	
1	185	190	1.4 *M* NaOH	110	120
2	155	180	2.9 *M* NaOH	105	110
3	165	185	6.2 *M* NaOH	95	95
4	190	190	10 *M* NaOH	95	110
5	160	170	Alcohol and ether	—	170
6	205	205	—	—	—

The experimental accuracy does not appear to be such that it is possible to distinguish between the different

concentrations of sodium hydroxide as regards their influence on the rate of diffusion of sodium chloride. It is rather surprising that concentrations between 1.4 and 10 *M* gave practically the same result. Only 0.3% of material was extracted from the chip soaked in ether, and the rate of diffusion of sodium chloride was unchanged. No conclusion can be drawn, therefore, whether the rate of diffusion would be increased by removing the fats and resins completely from the wood. More work will be necessary to explain definitely the action of sodium hydroxide on wood, whereby the rates of diffusion of sodium hydroxide and sodium chloride are increased in the lateral direction.

The results of the experiments at room temperature may be summed up as follows. In the longitudinal direction of black spruce heartwood, the three electrolytes diffuse at rates relative to each other in the order which would be expected from theoretical considerations—*i.e.*, hydrochloric acid the most rapidly, and sodium hydroxide and sodium chloride at nearly the same rates. In the lateral direction of the wood, sodium hydroxide diffuses the most rapidly because of its action on the wood, while sodium chloride and hydrochloric acid diffuse in the expected order of relative rates. The action of sodium hydroxide on the wood also increases the rate of lateral diffusion of sodium chloride to a degree similar to that of sodium hydroxide, and decreases the rate of longitudinal diffusion of both sodium hydroxide and sodium chloride.

Diffusion of Water into Air Dry Wood

The time required for an air dry chip, completely immersed in water, to sink was used as a measure of the relative rates of diffusion of water into different chips. The chips were of the same weights as those used in the experiments on the diffusion of electrolytes. Three series of experiments were carried out under the following conditions:—

(1) Open grained wood of about 15 annual rings per inch and a density of 0.40 of oven dry wood–at room temperature.

(2) Open grained wood of density 0.40 of oven dry wood —at 50° C.

(3) Close grained wood of 40-60 rings per inch and a density of 0.55 of oven dry wood–at room temperature.

The results of these experiments are given in Table VIII and are shown plotted in Fig. 5. The worst shape of chip for water diffusion, as indicated by the curve at 50°C., is shown in Fig. 3.

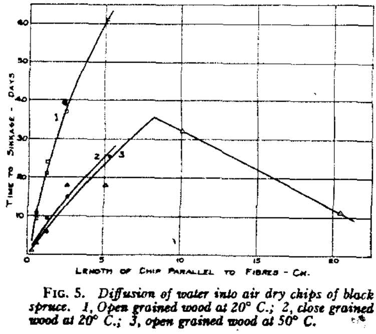

FIG. 5. *Diffusion of water into air dry chips of black spruce. 1, Open grained wood at 20° C.; 2, close grained wood at 20° C.; 3, open grained wood at 50° C.*

The data show that (i) raising the temperature from about 20 to 50° C. more than halved the time of sinkage, and (ii) the open grained wood required twice as long to sink as the close grained wood. A rise in temperature may cause three effects: *(a)* a decrease in the solubility of air in water, *(b)* more rapid diffusion of dissolved air molecules out of the chip, and *(c)* expansion of the air in the chip, which assists in its removal. Apparently the last two are predominant. The effect of the density of the wood is what would be expected, since a dense chip requires less water to diffuse into it before its density becomes greater than that of water, than is the case with less dense wood.

TABLE VIII

TIME REQUIRED FOR AIR DRY CHIPS TO SINK (T_s) IN WATER

Open grained wood at room temperature		Open grained wood at 50° C.		Close grained wood at 20° C.	
Chip length, cm.	T_s, days	Chip length, cm.	T_s, days	Chip length, cm.	T_s, days
0.64	11	0.32	1	0.76	4
0.66	9½	0.66	3	1.37	9½
1.27	24	1.29	6½	1.37	6
1.27	21	2.56	18	2.69	15
2.54	37	5.08	18	5.34	25½
5.13	61	10.1	32	—	—
—	—	20.5	11	—	—

The times required for sinkage obtained in these experiments with black spruce at room temperature are considerably greater than the values found by Richardson and Maass (1) for white spruce. The difference in results for these two varieties of spruce may be due largely to a difference in density of the wood.

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THE CAUSES OF THE CYTOLOGICAL RESULTS OBTAINED IN SPECIES CROSSES IN WHEAT[1]

BY W. P. THOMPSON[2]

Abstract

There are several possible causes of the absence or low frequency of many chromosomal and genetic types in crosses between 42- and 28-chromosome species of wheat. The actual effects of these possible causes are:

1. Pre-gametic causes — effect small. Among pollen grains which develop normally all chromosomal types are found but not quite in theoretical proportions; those with chromosome numbers intermediate between the 14 and 21 of the parents are somewhat deficient. Intermediate numbers are more abundant among retarded grains.
2. Gametic
   (a) Male
      (1) Outright abortion of pollen grains — 5 to 10%.
      (2) Retardation of development — 25 to 30%.
      (3) Failure of pollen germination — the above 25 or 35%, plus probably as much more.
      (4) Competition and selective fertilization — impossible to determine accurately but probably some effect.
   (b) Female — very little. Apparently nearly all female gametes are capable of functioning but 50% usually remain unfertilized.
3. Endospermic — abortion and abnormal development cause important effects particularly in relation to female gametes but they cannot be separated completely from possible direct embryonic effects.
4. Embryonic — about half the embryos abort before the seed is ripe and at least half the F_2 seeds usually fail to germinate. These are in part and may be wholly endospermic effects.

The gametic and endospermic influences are such as to reduce the proportion of plants with chromosome combinations intermediate between those of the parents.

The Problem

The chief genetic results of crossing *vulgare* (42-chromosome) wheats with emmer types (28 chromosomes) may be stated briefly as follows: A large proportion of the F_2 and an increasing proportion in later generations possess the combination of characters found in one or the other of the parental types. In comparison with expectations based on varietal crosses, there are relatively few plants showing various combinations of the numerous characters which distinguish the parents. Some plants do resemble one species in one or a few respects and the other parent in other respects. But in general the proportion with various recombinations of characters is far below Mendelian expectations.

Underlying these genetic results is a corresponding set of cytological findings. Most of the F_2 plants have one or the other of the parental chromosome numbers or some number approaching these. There are very few plants with chromosome numbers nearly intermediate between those of the parents, in comparison with expectations based on the functioning of all types of reproductive cells and the development of all types of embryos.

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The cytological results become still more striking when the mating capabilities of the chromosomes are determined (2, 3). In this respect it is found that the plants fall into two groups, (a) those with 14 pairs capable of mating (the number in the emmer parent) plus from 0 to 7 single unmated ones; and (b) those with more than 14 pairs, all such plants having enough single chromosomes in addition to make the total of mated and unmated 21 (the number of pairs in the *vulgare* parent). If, for example, there are 17 pairs in any F_2 plant, there are also four unmated single chromosomes (not 3, 2, 1, 0, or 5). Kihara (3) first showed that those in Group (a) tend to revert in later generations to the condition with 14 pairs only (no univalents), whereas those in Group (b) tend to revert to the condition with 21 pairs. A large number of conceivable combinations such as 15 pairs plus 0 to 5 singles, 16 pairs plus 0 to 4 singles, etc., very seldom appear, and the rare plants which possess such combinations are weak and sterile.

Any one of several conceivable causes might be responsible for these results in whole or in part. For example, at the second reduction division in F_1 all of the seven unmated *vulgare* chromosomes might tend to go to one pole and none to the other. Or certain kinds of gametes or zygotes might be inviable owing to unbalanced or unfavorable chromosome conditions. Furthermore the unbalance might express itself in various ways and at various stages in the life cycle. In the following pages an attempt is made to determine which of the possible causes produce any actual effect and what the exact effect is. Various investigators have published data bearing on the influence of particular causes. The conclusions from these scattered data are assembled together with certain unpublished observations. The different possible influences will be treated in the order in which they would operate during the life cycle.

I. Pre-gametic Causes

It is conceivable that the extra seven *vulgare* chromosomes which remain unmated at the first meiotic division do not segregate at random to the poles of the cell at the second division. If all seven should tend to go to one pole and none to the other the resulting gametes would tend to have 14 + 7 or 14 + 0 chromosomes. A very similar situation actually exists in rose hybrids (1). If it occurs in wheat it would largely account for the results which are to be explained, particularly the low frequency of plants with intermediate numbers.

With this possibility in mind Thompson and Armstrong (8) determined the chromosome numbers in many young pollen grains in order to decide whether grains with numbers intermediate between 14 and 21 are actually formed in expected proportions. This was done by direct counts at one of the two divisions which take place within the pollen grain to produce the male gametophyte.

The first counts were naturally made in stamens in which a large proportion of the pollen grains were actively dividing. The results are given in the first row of Table I. It will be observed that there is a small deficiency of grains

with intermediate numbers in comparison with the theoretical. This is shown graphically in Fig. 1. When it was found later that many of the F_1 grains are retarded in development, it was thought that the deficiency might be due to retardation of those with intermediate numbers. Stamens were therefore studied in which many of the grains had already divided, and only retarded ones were at the division stage. The results are given in the second row of Table I. It will be observed that they show no deficiency in the intermediate classes but rather a slight excess; but in view of the small number (40) studied it is doubtful whether the frequency distribution of retarded grains differs significantly from the theoretical. And since only about 30% of all grains are retarded in development, it appears that the retarded grains cannot entirely make up the deficiency of those with intermediate numbers which was found among those developing normally. All the data available therefore indicate a small deficiency of grains with intermediate numbers. But this deficiency is slight in comparison with that among grains which function and produce offspring, as shown in the next section (see Fig. 1).

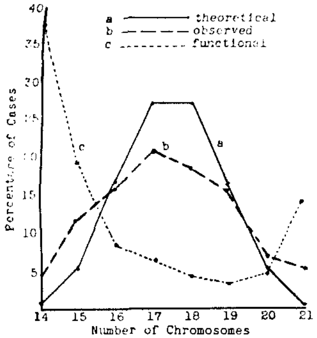

FIG. 1. *Frequency distribution of chromosome numbers in F_1 pollen grains; a, theoretical; b, observed in unretarded grains; c, functional.*

TABLE I

PERCENTAGES OF F_1 POLLEN GRAINS WITH VARIOUS CHROMOSOME NUMBERS

—	Chromosome numbers							
	14	15	16	17	18	19	20	21
Direct counts on normal grains	4.2	11.7	15.3	20.7	19.0	16.4	6.9	5.8
Direct counts on retarded grains	0.0	2.5	12.5	30.0	35.0	15.0	5.0	0.0
Functional grains	27.7	19.3	8.8	7.0	4.4	3.5	5.2	14.0
Theoretical	0.8	5.5	16.4	27.3	27.3	16.4	5.5	0.8

A subsidiary pre-gametic influence is the loss, through lagging, of univalent *vulgare* chromosomes at the meiotic divisions. Thompson and Hollingshead (10) found that in F_1 the approximate loss is one chromosome for every two pollen grains. Watkins (11) showed that a comparable loss occurs in 37-chromosome hybrid plants. The loss of chromosomes reduces the proportion of gametes with numbers higher than 14, and causes an excess of offspring with the lower numbers. This excess is reported by all investigators.

II. Causes Affecting the Gametes

It has frequently been suggested that one of the causes of the general cytological results is the failure to function on the part of the reproductive cells with intermediate chromosome numbers. In order to test this possibility Watkins (12), Sax (6) and the writer (9) independently undertook studies to determine the chromosome numbers of gametes which are capable of functioning. This was done by determining the chromosome numbers in plants which resulted from backcrossing the F_1 with the parents. The results of the different authors are in substantial agreement. Some of the writer's are given in Table I and are represented graphically in Fig. 1. Similar results have recently been published by Kihara (5).

It is clear that the great mass of pollen grains with intermediate numbers must have failed to produce offspring. The curve which represents the theoretical frequency distribution of the various types of pollen grains must be inverted to produce the curve for the grains which actually functioned.

The results for female gametes were similar but a larger proportion with intermediate numbers functioned than in the case of the male. Kihara (5) has recently stated that when F_1 is backcrossed with emmers, the results are nearly the same for female gametes as for male but, when it is backcrossed with *vulgare*, eggs with intermediate chromosomes function in nearly the expected proportions.

The inability of the great majority of the pollen grains to produce offspring may be due to several causes. These will now be considered.

(a) Death of Pollen Grains

A number of observers have reported that many of the mature F_1 pollen grains entirely lack protoplasm and that others have very little. The percentage of such grains is usually given as from 10 to 15. It is to be noted that there are various gradations between grains which are completely empty and those which appear perfectly normal, and that it is therefore impossible to draw a definite line between those which are clearly capable of functioning and those which obviously are not. In any case 10 to 15% is far less than the deficiency of functioning grains as determined by chromosome counts on backcross plants.

(b) Retardation of Development

Normally developing pollen grains of wheat enlarge greatly a short time after the completion of the meiotic divisions. This causes the presence of a large vacuole, the cytoplasm becoming restricted to a thin peripheral layer. The cytoplasmic layer then gradually thickens until the vacuole completely disappears some days before maturity. The first division of the pollen nucleus takes place while the vacuole is still large, and the second (the products of which become the sperms) occurs after the vacuole has disappeared.

In the F_1 all grains develop normally until the large vacuole is present (Thompson and Armstrong (8)). In a small percentage no further develop-

ment takes place; the cytoplasm not only fails to increase but gradually disappears. These are the ones which will be devoid of contents when the stamen is mature. Most of them can be recognized at the time the normal grains are undergoing the first gametophytic division.

In other grains the growth of the cytoplasm is slow. These lag farther and farther behind the normal. In different grains there are various rates of cytoplasmic increase and many seem to cease developing entirely at various stages. At the time when the first division is occurring in the normal grains only about 60% have the normal amount of cytoplasm, 25 to 30% show some degree of reduction, 6 to 12% have very little and about 1% are quite empty. By the time the stamen is mature the percentage of empty grains has risen to about five, while that of grains with a reduced amount of cytoplasm has decreased slightly.

There is a corresponding retardation in nuclear development which is evident at all stages. For example, when the normal grains have three nuclei many others have not yet divided once, and still more have only two nuclei. At maturity of the stamen, when normal grains have two sperms and a tube nucleus, about 5% are quite empty, 5% have only one nucleus, 10 to 15% have two, and another 10 to 15% have three nuclei but have not yet organized sperms. The grains with the retarded nuclear development are for the most part those with less than normal cytoplasm, although a small percentage which appear normal in cytoplasmic content have only two nuclei or no sperms.

It was shown in a previous section that pollen grains which are retarded in development are more likely to have chromosome numbers intermediate between 14 and 21 than are those which develop normally. This is evident from the data given in Table I. Presumably those which never divide at all, including those which lose their cytoplasmic contents, also have intermediate numbers.

(c) Pollen Germination

The abnormalities described in the last section leave 60 to 70% of the pollen grains in mature F_1 stamens apparently normal in cytoplasmic and nuclear development. About 10% are either entirely empty or have very little cytoplasm with a single nucleus and are evidently incapable of functioning. The remaining 20 or 30% show various amounts of cytoplasm and various stages of nuclear development. It seems probable that they also would be unable to germinate. Moreover it is possible that some of the grains which appear normal may be injuriously affected by chromosome conditions in ways which produce no visible effect. Tests of the germinating capacity of the pollen were therefore undertaken.

Watkins (11) has studied the germination of F_1 pollen of *vulgare* × *turgidum* and found that from 5 to 30% germinated depending on conditions.

Thompson and Armstrong (8) found it impossible to secure reliable results on the germination of any kind of wheat pollen in the laboratory. They were therefore compelled to make their observations on pollen in contact

with stigmas. It was found that in conditions in which 80 to 90% of parental pollen germinates none of the grains with any reduction in the amount of cytoplasm or with retarded nuclei are able to germinate. Moreover a large percentage of the apparently normal grains also fail to germinate. Altogether not more than 15% of all grains germinated on F_1 stigmas in our experiments. It must not be concluded that under entirely natural conditions the germination would be as low as this but it is evidently considerably lowered even for normal looking grains in comparison with parental pollen.

(d) Competition Between Gametes and Selective Fertilization

Pollen grains with certain chromosome numbers may be able to develop and germinate but their tubes may be handicapped, by slow growth, in competition with grains which have more favorable chromosome combinations. This possibility cannot be tested directly owing to the nature of the feathery, much-branched wheat stigma, which is attached directly to the ovary. But from what is known of similar situations in other plants (for example $n + 1$ pollen in *Datura*), it may be important. The only direct evidence bearing on the point in wheat is that, at 10 hr. after pollination, one in every four of the germinated F_1 pollen grains has developed only so far as to protrude the tip of the tube, the rest of the germinated grains having by this time produced well developed tubes. On the other hand among parental pollen grains only 1 in 14 is so retarded.

Kihara (5) has recently published results which indicate that when F_1 plants receive emmer (14-chromosome) pollen, it is chiefly 14- to 16-chromosome eggs which function and produce offspring, whereas when the same F_1 plants receive *vulgare* (21-chromosome) pollen it is chiefly 17- to 21-chromosome eggs which produce offspring (Table II). A strong selective effect is thereby indicated. It must be remembered, however, that when F_1 is backcrossed to *vulgare* the germination of the seeds is poor, and that the low frequency of backcross plants from 14- to 16-chromosome eggs may be due, in part at least, to incomplete germination. The writer's results, involving only a small number of plants, did not indicate such a selective action.

Kihara also stated that when emmer plants receive F_1 pollen it is chiefly 14-chromosome pollen grains which function and produce offspring, whereas when *vulgare* plants receive the same F_1 pollen 21-chromosome grains function as frequently as those with 14 (Table II). But again it must be remembered

TABLE II

FREQUENCY OF F_1 GAMETES WITH VARIOUS CHROMOSOME NUMBERS, WHICH PRODUCED OFFSPRING IN BACKCROSSES. (DATA FROM KIHARA (5))

Chromosome number	14	15	16	17	18	19	20	21	Total
F_1 × *vulgare*	4	3	2	6	9	5	2	0	31
F_1 × *durum*	37	16	15	4	1	3	1	4	81
T. vulgare × F_1	6	7	2	2	4	2	1	8	32
T. durum × F_1	12	8	2	0	1	0	0	0	23

that in emmer × F_1 the germination of the seeds is poor and the low frequency of plants from 21-chromosome pollen grains may be due, in part at least, to poor germination. It is also conceivable that in this type of backcross (parents × F_1) the 14-chromosome tubes grow better in 28-chromosome stigmas and 21-chromosome tubes grow better in 42-chromosome stigmas (14). If that is the case, 14-chromosome grains would function most frequently when emmer is the female parent used and 21-chromosome grains would function better when *vulgare* is the parent. Opposed to this conception is the fact that in the reciprocal type of backcross (F_1 × parents) there is a similar difference in the eggs which function depending on the parent used. But in this case the nature of the stigma can play no part since all stigmas are alike (F_1).

(c) Abortion of Female Gametes

All investigators agree that less than 50% of the F_1 flowers set seed when they are self-pollinated and the percentage often falls as low as 15. This has been regarded as the result of the abortion of embryo sacs corresponding to the abortion of pollen. Earlier reports of the results of backcrossing F_1 to the parents would lead to the same conclusion. On the other hand Watkins (11) reported that F_1 flowers which receive parental pollen set seed in almost as high a proportion of cases as parental flowers in crosses between pure lines (87% for F_1; 90% for pure lines). This would indicate that almost every female gamete is able to function successfully. This result is confirmed by Kihara (5) who obtained up to 90% seed setting on F_1 when the work was done very carefully. It therefore appears well established that there is very little abortion of embryo sacs and that low seed setting when F_1 is selfed is due to the failure of fertilization or to something which happens subsequent to fertilization.

This conclusion is supported by Kihara's (4) microscopic examination of ovules collected two or three days after the flowering time. He found that less than 2% of the embryo sacs were degenerate. Although only 41% contained embryos, the remaining 57% were healthy and had apparently escaped fertilization. Watkins (11) has reported similar findings for certain F_3 plants which were almost as sterile as F_1.

The abortion of female gametes is therefore not an important factor in the general cytological situation.

III. Causes Affecting the Endosperm

It is well known that the seeds borne on interspecific hybrids are commonly small and wrinkled or shrivelled. In such cases the amount of endosperm is usually less than in the parent seeds and it is almost invariably abnormal in appearance or texture.

Watkins (12) and Thompson and Cameron (9) showed that in wheat there is a correlation between the abnormal endosperm condition and chromosome numbers. If the 14-chromosome parent is female the F_1 endosperm is

wrinkled, but in the reciprocal cross the endosperm is plump. The chromosome numbers in the endosperm are quite different in these two reciprocal crosses, owing to the fact that the tissue develops from a fusion of one male and two female nuclei. When the 14-chromosome parent is female the wrinkled endosperm has only one set of the extra seven chromosomes contributed by the male *vulgare* gamete (49 in all); in the reciprocal cross it has two sets of the seven (56 in all).

Thompson (7) carried the study further and determined the chromosome conditions in a large number of backcross and F_2 seeds of various degrees of shrivelling. He found that the endosperm is wrinkled or shrivelled whenever it is (a) haploid for all or many of the extra seven *vulgare* chromosomes, or (b) diploid or triploid for only a part of the seven. The farther the chromosome situation departs from the normal complete absence or complete triploidy of the seven *vulgare* chromosomes the severer is the shrivelling. A doubled or tripled condition of part of the set of seven causes a lack of balance which prevents normal endospermic development.

Such abnormal endospermic conditions must occur precisely in those seeds from which the missing chromosomal types of plants would have to develop. For example, a plant with 18 pairs of chromosomes (and no univalents) would have to develop from a seed which had four of the extra seven represented three times in its endosperm and the remaining three quite unrepresented. This highly unbalanced chromosome condition would cause the abortion of the endosperm. Other missing chromosomal types would have to develop from seeds with similar unbalance in the endosperm although, of course, the unbalance would not always be so extreme.

In extreme cases it may be expected that the endosperm could not develop at all and consequently the seeds could not be formed. In the absence of microscopic studies it is impossible to say in what proportion of cases this occurs. In less extreme cases the endosperm would develop slowly or abnormally and would be shrivelled. The shrivelling lowers the germination and thus eliminates the chromosomal types of zygotes involved. There are many published data on the poor germination of hybrid seeds in these crosses. Thompson and Cameron (9) classified their seeds according to the degree of wrinkling and showed the expected high correlation between wrinkling and poor germination. In backcrosses the percentage of seeds which germinate depends on the direction in which the backcross is made; in some cases it is only 35%. In direct crosses probably never more than 50% of the seeds formed on F_1 plants by self pollination are able to germinate.

It might be supposed that the elimination of gametes at pre-fertilization stages as described in previous sections would reduce the possible endospermic effect to small proportions, since few gametes might be left to eliminate. But it must be remembered that female gametes are much less affected than the male by pre-fertilization influences; nearly all of them, in fact, are apparently able to function. And the endospermic influence would be particularly

effective in relation to female gametes, since the female contribution (and therefore the unbalancing effect of any extra chromosomes which it contains) is doubled in the endosperm.

IV. Causes Affecting the Embryos

The unbalanced chromosome condition which injuriously affects the endosperm may also affect the embryo independently of the endosperm. It is to be expected, however, that the triploid endosperm would be more injuriously affected than the diploid embryo since the unbalance would be greater. In agreement with that expectation is the fact that shrivelled grains, if they germinate at all, may produce perfectly healthy plants. If there is a direct effect on the embryo it would be difficult to distinguish from the effect produced through the abnormal endosperm except perhaps at early stages.

Kihara (4) has provided some data on the abortion of embryos. In a microscopic study of ovules collected two or three days after pollination he found that 41% of the F_1 embryo sacs contained embryos. But in fully comparable mature ears only 16% of the flowers had produced seeds. Therefore more than one-half of the embryos had aborted. This means that approximately 25% of all flowers are sterile because embryos which are formed in them fail to develop. To this amount of zygotic elimination must be added that which is involved in failure of the seeds to germinate.

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THE GENERA *HALICTUS* AND *ANDRENA* IN WESTERN NOVA SCOTIA[1]

By C. E. Atwood[2]

Abstract

This paper deals principally with the classification of the bees of the genera *Halictus* and *Andrena* collected during a five-year project on the pollination of the apple in the Annapolis-Cornwallis Valley, Nova Scotia. Keys for the separation of the species concerned are given, together with figures of the eighth and ninth abdominal sterna of the males, which were found very useful in separating related species. One new species, *Andrena kalmiae* Atwood, and the true male of *A. ceanothi* Vier., are described, and a re-description of *Halictus arcuatus* Rob. is given, including a summer form which is presumably sterile. The taxonomic section is prefaced by brief notes on economic importance and biology of the genera.

Introduction

The study of the two genera of bees, *Halictus* and *Andrena*, as represented in western Nova Scotia was begun in connection with an investigation of the problem of apple pollination in the Annapolis-Cornwallis Valley, conducted in the seasons 1928-1932 by the Department of Agriculture, Ottawa, the work being under the immediate direction of Dr. W. H. Brittain.

When counting the numbers of various insects which visited apple bloom, it was clear that the bees of the genera *Halictus* and *Andrena* played a leading part in apple pollination. Accordingly, biological and taxonomic studies appeared necessary for intelligent interpretation of such counts and presentation of the results. It was eventually decided to include in the present study all the species of these genera found in the region investigated, whether taken on apple or not, in order to make the list as complete as possible. The results of studies on the material collected during the summers in which the project was carried on are presented herewith. A considerable number of specimens of other genera of bees, and other insects, were also taken; these have been placed in the National Collection at Ottawa and await further study. The list of *Halicti* and *Andrenae* cannot be considered as complete for the province. Several species reported from there have not been taken by the writer. Some of these reports were based on incorrect identifications. It is hoped, however, that the keys, descriptions, and drawings given will enable anyone to identify the great majority of species of these genera that may be taken in Nova Scotia.

Economic Importance

In the apple growing districts of Nova Scotia, four genera of bees are chiefly concerned with the pollination of the bloom and consequent production of the crop. Three of these, *Bremus*, *Halictus*, and *Andrena*, are native,

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while the fourth is represented by the domesticated *Apis*. Owing to the losses caused to beekeepers by spraying and dusting for orchard pests, very few honeybees are kept in the Annapolis-Cornwallis Valley at this time so that these insects are a negligible factor in the pollination of apple over most of this area. The evidence also seems to show that *Bremus* has decreased in numbers in the orchard region, and at present also plays a very small part in orchard pollination. Consequently, the other two groups are chiefly responsible for the pollination which takes place, as they seem to have suffered very little from poisoning. This is not an entirely satisfactory situation because these bees are more sensitive to temperature and light than *Bremus* and *Apis* and fly only on comparatively warm and sunny days, so that a long period of dull weather during bloom may result in lack of sufficient pollination unless honeybees are introduced. On the whole, however, their efforts must be considered successful to a large degree because the crops which are produced are the result of their activities. In addition to apple, these bees also act as pollinators for cherry, plum, pear, and the various smaller fruits such as raspberry, strawberry, and gooseberry, as well as numerous wild and cultivated flowers. Both from the economic and from the aesthetic standpoint their importance can hardly be overestimated.

Biology

The life histories and nesting habits of the genus *Andrena* have been studied in Europe by various entomologists. Perkins (5) gives an excellent account of some British species and their parasites. They are entirely solitary, each female making her own nest, although the holes may be very close together in favorable places. There may be two broods per year, but all the Nova Scotian species appear to have only one. This brood may appear in early spring, as in the case of *A. carlini*, or in the fall, as in *A. hirticincta*.

The burrows of those observed in Nova Scotia were generally constructed among grasses or other low herbage rather than on bare soil, and were rather difficult to find. They varied in length from a few inches to some two feet, being as a rule deeper on sandy soil. The pollen was stored in some cases as a pellet, in others as a very stiff paste of mixed nectar and pollen. Several species of *Nomada*, a genus known to be parasitic on *Andrena*, were taken in the region, but their relation to particular species of *Andrena* has not yet been traced. Nova Scotian bees of several genera are also attacked by *Strepsiptera*, *A. thaspii* being apparently most susceptible.

Although there has been very little written about the nesting habits of American *Halicti*, European species have attracted considerable attention. It has been generally agreed that the spring brood of *Halictus* consists of females only and that males do not appear until midsummer or later, but various opinions have been expressed regarding the details of the different broods. Fabre (2) concluded that the spring brood consisted of overwintered females who made the nests and laid the first eggs; that these were followed by a parthenogenetic brood of females; and that these laid eggs which gave

rise to a fall brood of males and females, the latter going into hibernation. The spring females were also considered to live until fall and to act as guardians over the cells provisioned by their daughters. Armbruster (1) stated that there were two parthenogenetic generations, the first producing a summer brood of females only, this again giving rise parthenogenetically to a fall brood of males and females. The latest work is that of Stöckhert (10), who differs from both of the foregoing. He found that the females of *H. malachurus* spend the winter in hibernation and emerge early in the spring, immediately beginning to clean out the burrow or to dig new ones. The number of summer broods differ; there may be one, two, or three. In the species which have three broods, there is a primitive type of social organization. The first eggs laid by the spring female develop into females which are smaller than their mother and often differently sculptured. These visit flowers and gather pollen and nectar to provision the nest cells while the original mother lays more eggs, of which some produce females, the others males. The summer brood never lay eggs and are never fertilized by males, and therefore correspond to the workers of *Bremus*. In the fall the mother, the males, and the sterile females die, while the fertilized fall females hibernate, several usually being found in one burrow.

The habits of the species observed in Nova Scotia seem to be very similar to those recorded for the German species. *H. zonulus* and *H. provancheri* appear to have only two generations. *H. rubicundus* appears to have a sterile brood of some six females. *H. arcuatus* and *H. viridatus* have the most highly organized social system, apparently quite similar to that of the three-brooded species noted by Stöckhert. They appear very early in the spring and begin making and provisioning their nests. *H. arcuatus* does not hibernate in its summer hole and therefore must dig a new nest, while *H. viridatus* may simply clean out the previous year's nest in which it has spent the winter. While most of the spring nests are occupied by a single female, many of them may contain two, three, or four females. It is remarkable that although *H. arcuatus* does not hibernate in its summer tunnel, two females have been found excavating a new hole together in May. In the case of *viridatus* several females are commonly found in the hibernation tunnel, but in spring one usually retains the winter hole while the others make new ones. This procedure is by no means invariable, however, as indicated above. When two or more females occupy the same hole, one is frequently seen standing guard at the entrance.

The first adults of the summer broods begin to emerge from the pupal state during the latter part of June; these are sterile females. The males do not appear until nearly a month later. It was not found possible to establish a definite line between the spring females and the sterile second generation as regards external characters, but in *H. arcuatus* a number of summer females are smaller and have a proportionately longer dorsal space on the propodeum, as well as slightly different sculpturing. In *viridatus* a marked difference in size is often seen among the inhabitants of a single nest; differences of puncta-

tion occur also, but none of these have been definitely associated with any particular brood. Stöckhert's observations were checked by dissection of the female reproductive system. This has not been done as yet with the Nova Scotian species under consideration, and would undoubtedly throw additional light on the subject. The evidence would seem to indicate, however, that the two last-mentioned species show an elementary social system, with a primitive worker caste, and some degree of division of labor and co-operation among the inhabitants of a nest.

Taxonomy

The taxonomic literature on these two genera is scattered through many journals and books and is written in several languages. Most of the earlier attempts to classify the bees were made by Europeans; among the important contributions being those of Schmiedeknecht (7), Friese (3), and Smith (8, 9.) The latter described many of the North American species but his descriptions are usually too vague to make exact identification certain without comparison with the types. The others have proposed classifications of the bees as a whole, each differing from the others in his ideas as to the relationships between the various groups. In America, Cresson, Ashmead, and Robertson have contributed many important papers on the *Apoidea*, and Robertson and Viereck have proposed schemes for the division of *Halictus* and *Andrena* into subgenera.

Because of the large number of species, the variability of characters, and the inadequate descriptions of the earlier writers, the nomenclature for these genera is still in a very confused state and must remain so until a careful revision of the North American species is completed. For this reason, a paper dealing with a local fauna must contain some names which are more or less tentative, and this is true of the present list.

Because of the slight and inconspicuous characters distinguishing some species, it is often very difficult to indicate differences in a key or verbal description. Morice (4) in 1899 pointed out that the genital armature and the eighth ventral segment in *Andrena* were of considerable value in distinguishing closely allied European species of that genus. These characters have been examined in the Nova Scotian species under consideration, and an attempt made to illustrate them by semidiagrammatic drawings. In many cases examination of these is unnecessary for the separation of the species mentioned in this paper, but a drawing of such a character defines a species in a more precise way than the comparative differences given in keys, and makes it possible to distinguish it from a related species, no matter where the latter may occur.

In some cases it has been possible to secure only one sex of a species, and consequently the missing one does not appear in the keys. Also, several unidentified species have been omitted completely as they are represented by only one or two specimens which could not be recognized from descriptions

or by comparison. They may be new species but it is better to wait until more material is secured, so that a series is available for study. In the meantime they will be deposited in the National Collection at Ottawa.

One of the chief difficulties in dealing with these bees is the proper association of sexes, as the males and females are often extremely unlike each other. The securing of nests and their inhabitants, the taking of couples *in copula*, and data on time of appearance and flowers visited may help to solve this difficulty, and a special effort was made to secure as much information of this type as was possible. The taking of populations from nests also permits the limits of variation within the species to be studied; the lack of study of this subject has led to the naming of many species on insufficient grounds.

In regard to the synonymy of the species dealt with, the writer is greatly indebted to Miss Sandhouse, who has compared some of the material with European specimens and established their identity.

Halictus Latreille

Key to Species

Females

1. Portion of cubital vein between second and third transverse cubital veins not obsolescent; abdomen with heavy apical fasciae of appressed pubescence 2
   Portion of cubital vein between second and third transverse cubital veins obsolescent; abdomen with fasciae basal, indistinct, or absent 3
2. Head, thorax, and abdomen black; propodeum rugulose; mesonotum and abdomen closely punctate .... ***rubicundus*** (Christ.) Kby.
   Head, thorax, and abdomen greenish; clypeus purplish; propodeum, mesonotum, and punctate abdomen as above .... ***provancheri*** D.T.
3. Head and thorax greenish or bluish 13
   Head and thorax black 4
4. Mesonotum and abdomen very closely punctate, lustreless, usually with a bluish bloom; second, third and often fourth abdominal terga with heavy basal fasciae of appressed pubescence; posterior edges of terga thick; propodeum rugulose or granular, roughest at sides; hind tibial spur with eight or more short, rather broad and blunt teeth 5
   Mesonotum and abdomen polished and shining between punctures; teeth of tibial spur either fine serrations, or fewer in number than above, and long; posterior edges of terga thin 6
5. Propodeum smooth at posterior edge of dorsal space .... ***coriaceus*** Sm.
   Propodeum rough at posterior edge of dorsal space .... ***athabascensis*** Sandh.
6. Head conspicuously short and broad; teeth of tibial spur fine serrations; propodeum short, coarsely rugose; first abdominal tergum finely and sparsely punctate; complete or interrupted basal fasciae usually present .... ***arcuatus*** Robt.
   Head not conspicuously short; clypeus at least slightly produced 7
7. Propodeum smooth at posterior edge of dorsal space; first abdominal tergum very finely and sparsely punctate; about four long tibial teeth 8
   Propodeum rough (rugose or granular) from base to posterior edge of dorsal space 9
8. Second and third abdominal terga without patches of white pubescence on sides; mesonotum very finely and closely punctate .... ***foxii*** Robt.
   Second and third abdominal terga with patches of white pubescence on sides; mesonotum with punctation slightly more coarse than above .... ***macoupinensis*** Robt.
9. First abdominal tergum impunctate; mesonotum strongly punctate; propodeum coarsely rugose, dorsal space bounded posteriorly by a salient rim; first recurrent vein practically continuous with second transverse cubital vein; tibial spur with about six long teeth .... ***pectoralis*** Sm.
   First abdominal tergum at least finely punctate 10

10. First abdominal tergum strongly punctate; second, third and usually fourth with broad, complete or incomplete basal fasciae; propodeum coarsely rugose 12
    First abdominal tergum finely and sparsely punctate; fasciae very thin, or on sides of terga only; propodeum rather finely rugulose or granular 11

11. *Abdomen not fasciate or with small incomplete fasciae at sides; tibial spur with about six rather long sharp oblique teeth; rugae of propodeum rather close together; posterior edge of dorsal space decidedly roughened* .... *rufitarsus* Zett.
    Abdomen with thin apical fasciae of sub-erect, pale rusty hairs, usually complete on one or more terga; propodeum long, rugae rather far apart; posterior edge of dorsal space almost smooth; tibial spur with about four long, pointed oblique teeth .... *quebecensis* Cwfd.

12. First abdominal tergum regularly and closely punctate, rather dull; tibial spur with six or more conspicuous stout teeth .... *leucozonius* (Schrank.)
    First abdominal tergum sparsely punctate toward centre, more closely toward sides, polished and shining; tibial spur finely serrate .... *zonulus* Sm.

13. Thorax and abdomen yellowish green; mesonotum distinctly and very closely punctate; first abdominal tergum finely and closely punctate; second, third and fourth terga practically covered with appressed, felt-like, yellowish pubescence, propodeum closely rugulose .... *pilosus* Sm.
    Abdomen black, or with a faint brownish tinge; pubescence somewhat more sparse and less felt-like than above 14

14. Mesonotum coarsely punctate 15
    Mesonotum finely but distinctly punctate, abdomen black, shining .... *viridatus* Lov.

15. Abdomen blackish opaque, posterior margins of terga not conspicuously testaceous, wings yellowish .... *cressonii* Robt.
    Abdomen brownish, shining, posterior margins of terga broadly testaceous; wings clear .... *nubilus* Lov.

Males

1. Abdominal segments with apical fasciae; closely punctate; clypeus with a broad transverse yellow mark at apex; hind tibiae and tarsi mostly yellow; propodeum closely rugulose 2
   Abdominal segments without distinct apical fasciae, at most a thin band of sub-erect hairs along apical margin 3

2. Head, thorax, and abdomen greenish .... *provancheri* D.T.
   Head, thorax, and abdomen black .... *rubicundus* (Christ.) Kby.

3. Thorax, greenish, bluish or purplish; dorsal space of propodeum rugose; abdomen black or brownish 12
   Thorax black; dorsal space rugose or rugulose, sometimes smooth at posterior edge of dorsal space 4

4. Anterior portion of clypeus flattened or concave, shining and polished, almost or entirely without hairs or punctures, a broad transverse yellow spot at apex; first abdominal tergum closely punctate 5
   Anterior portion of clypeus transversely rounded, more or less punctate, and with a distinct covering of hairs; yellow spot present or absent 6

5. Tip of mandible when closed reaching practically to base of opposite mandible; face very broad; form robust; basal fasciae of abdomen mostly complete. . *coriaceus* Sm.
   Tip of mandible when closed reaching only about three-fourths of distance to base of opposite mandible; smaller, form less robust; basal fasciae mostly incomplete, tarsi reddish .... *athabascensis* Sandh.

6. Sixth sternum of abdomen with an entire and evenly rounded posterior margin; no conspicuous bands or tufts of hair on that segment 8
   Sixth sternum with distinct bands or tufts of hair 7

7. Sixth sternum of abdomen with two prominent tufts of hair along its longitudinal axis, postero-lateral margin reflexed; face broad; first abdominal tergum sparsely punctured toward centre, more closely toward edges; posterior tarsi brownish .... *zonulus* Sm.
   Sixth abdominal sternum with two dense converging bands of hair, which meet about half-way from apex to base, giving it a notched appearance; clypeus somewhat produced; first abdominal tergum evenly punctate; posterior tarsi yellow .... *leucozonius* (Schrank.)

8. Dorsal space of propodeum rugose, bounded posteriorly by an elevated and roughened ridge.......... 9
   Dorsal space rugulose at base, smooth at posterior edge, without a sharp rim, clypeus with a pale yellow or whitish mark.......... 11
9. Antennae not conspicuously longer than thorax, joint three about equal to joint four; clypeus black; mesonotum strongly punctate; propodeum with few rugae; abdominal terga with numerous short, light, sub-erect hairs......*pectoralis* Sm.
   Antennae conspicuously longer than thorax, joint three not more than one-half as long as four; propodeum with numerous irregular rugae.......... 10
10. Clypeus produced; mesonotum finely punctate; first abdominal tergum very finely and sparsely punctate; hind tarsi brownish..........*rufitarsus* Zett.
    Clypeus hardly produced; mesonotum and first tergum strongly punctate; hind tarsi whitish..........*arcuatus* Robt.
11. Form slender, conspicuously longer than thorax..........*foxii* Robt.
    Form not slender, antennae not conspicuously longer than thorax...*macoupinensis* Robt.
12. Head almost black, thorax dark purplish..........*consonus* Sandh.
    Head and thorax distinctly green or bluish.......... 13
13. Clypeus with a yellow mark; mesonotum finely and closely punctate; distal end of posterior femora and both ends of posterior tibiae bright yellow; abdomen greenish..........*pilosus* Sm.
    Clypeus without a yellow mark; mesonotum not very closely punctate; abdomen black or brownish.......... 14
14. Punctures of mesonotum sparse, coarse; face densely covered with white felt-like pubescence.......... 15
    Punctures of mesonotum fine; face usually with sparse appressed hairs.....*viridatus* Lov.
15. Antennae black or dark brown, abdominal terga without a broadly testaceous margin..........*cressonii* Robt.
    Antennae light yellowish brown beneath; posterior margins of abdominal terga broadly testaceous..........*nymphaearum* Robt.

H. arcuatus Rob.*

H. arcuatus Robertson. Trans. Am. Ent. Soc. 20: 145; 1893.

Female

Black; head short and broad, clypeus coarsely, sparsely, and irregularly punctate, a very conspicuous fringe of yellowish hair on apex; mandibles blunt, red at tip, with a strong preapical tooth; face closely punctate, somewhat roughened, with sparse, whitish erect hairs, antennae black or dark brown, basal joint shining.

Mesonotum shining, strongly and regularly punctate, surface finely roughened, pubescence gray or light brownish, sparse except along edges; scutellum with a wide area along axis closely punctate and roughened, and a shining almost impunctate space each side, edges of sclerite closely punctate, hairs similar to those of mesonotum but longer; metanotum rough, mostly covered with appressed dull gray pubescence with long hairs over; dorsal space of propodeum shining, very strongly rugose, with about 15 longitudinal ridges, which extend to the posterior margin and make it very rough in the central area; posterior margin slightly rounded; lateral fringe of hairs thin; sides of propodeum striate around edges, smooth and tessellate in centre; wings slightly tinged with brownish, veins and stigma clear yellow testaceous, second submarginal cell nearly as long as third, tegulae brownish or nearly all black; legs black, brown, or reddish, tarsi reddish, pubescence gray, or yellowish toward apical joints, especially on inner side of tarsi; hind tibial spur with about six short, sharp, oblique teeth.

**A full description of this species is given in order to distinguish the spring from the summer form.*

First abdominal tergum polished and shining, finely and sparsely punctate, following segments more closely punctate, especially on bases; posterior borders of terga brownish testaceous; second, third, and usually fourth with patches of white appressed pubescence on sides, which may extend completely across segment or be missing altogether from one or more; third and fourth, and sides of first and second with sparse semi-erect yellowish or grayish hairs, fifth densely covered with yellowish or light brownish hairs about rima. Length 7-9 mm.

What appears to be a summer form of this species has been taken in great numbers on sumac (*Rhus*) and rose during July and August, and also from nests in the only community of this species which was found. It is smaller, about 6-7 mm.; the dorsal space of the propodeum is long, more rounded behind and more finely and irregularly rugose, almost reticulate, and the patches of appressed whitish pubescence on the abdomen are usually small, seldom present on the fourth segment and sometimes practically absent.

Male

Black; face broad, densely clothed with white, felt-like pubescence, clypeus with a more or less distinct pale yellow mark anteriorly, sometimes almost completely absent; antennæ very long, black or brown, with constrictions between joints; mesonotum like female; scutellum punctate all over, a rounded elevation on each side of longitudinal axis; metanotum closely rugulose, with sparse erect hairs only; propodeum shining, dorsal space very coarsely rugose, posterior space and sides more finely rugulose or reticulate, pubescence of thorax gray or brownish; wings clear hyaline, veins and stigma dark testaceous to brown; legs black, tibial spurs and first tarsal joint light yellow, the latter with a black or brown distal end, other tarsal joints black or brown with a varying amount of yellow at base; abdomen slender, black, shining, closely and finely punctate, with patches of white appressed pubescence on second and third segments. Length 7-8 mm.

The nearest eastern species to this is apparently *H. truncatus* Rob., from which it is distinguished by the punctate first abdominal segment.

180 females, 31 males, numerous other specimens exchanged. Hants, Kings, and Lunenburg counties, May 20 to August 23. Common, especially on Rosaceae and Compositae.

Andrena Fabricius

Key to Species

Females

1. Mesopleura with a conical or somewhat keel-shaped projecting process; clypeus strongly punctate with an upturned rim along its anterior edge....... ***persimulata*** Vier.
   Mesopleura without such a projecting process.................................... 2
2. Facial line distinctly longer than transfacial line; facial fovea narrow; second tergum depressed posteriorly less than half of its length; first tergum sparsely punctate, minutely tessellate between punctures; pubescence mostly pale, anal fimbria brown.. ***bradleyi*** Vier.
   Facial line shorter than transfacial line.. 3

3. Dorsal space of propodeum coarsely rugose, usually with distinct longitudinal rugae, sometimes almost reticulate, generally bounded posteriorly by a transverse ridge; third joint of antennae shorter than, or at most equal to, fourth and fifth together; mesonotum with strong clear-cut punctures, which stand out conspicuously from the minute tessellations between them; abdomen strongly and rather closely punctured.. 23

   Dorsal space of propodeum rugulose, or plicatulate at base, triangular, never with strong elevated ridges running from base to posterior edge, usually defined by an impressed line or absence of hair, and continued to a point on the posterior face; never separated from posterior face by a raised transverse ridge; third joint of antennae generally longer than fourth and fifth together; mesonotum with punctures partially obscured by the roughening between them.................. 4

4. Hind tibiae and tarsi bright yellowish or ferruginous; abdominal terga distinctly punctate, strongly roughened; clypeus with a narrow impunctate space forming a raised line down its centre, this area tessellate..................*wilkella* (Kby.) Ill.

   Hind tibiae and tarsi at most dull reddish brown.................................. 5

5. Hairs of tibial scopa dark brown or black.. 6

   Hairs of tibial scopa light gray to fulvous.. 10

6. Abdominal terga, except the apical two, without conspicuous long hairs.............. 8

   Terga conspicuously clothed with long, more or less erect, hairs..................... 7

7. Pubescence of head, thorax and dorsum of abdomen except anal fimbria yellowish, erect, dense; clypeus closely punctate with a narrow, roughened, median impunctate space..*hirticincta* Prov.

   Pubescence of mesonotum and at least the first two abdominal terga bright fulvous; that of face, cheeks, part of pleura and apical terga black or dark brown; clypeus sparsely punctate, median impunctate space wide, polished, shining *milwaukeensis* Graen.

8. Face, cheeks, and pleura without black or dark brown hairs; face coarsely punctate with a narrow shining impunctate space...............................*vicina* Sm.

   Face and cheeks with some dark brown or black hairs; pleura with a patch of dark brown or black hairs.. 9

9. Face with coarse irregular punctures, and a distinct median impunctate space.*carlini* Ckll.

   Face with rather small regular punctures, no median impunctate space....*regularis* Mall.

10. Face with some black hairs; clypeus closely punctate with a narrow triangular or T-shaped median space; abdominal terga with long, silky, rather sparse gray hairs, not forming definite transverse bands............................*frigida* Sm.

    Face without black hairs.. 11

11. Second, third, and fourth abdominal terga with complete apical fasciae of dense appressed ochraceous hairs; first tergum impunctate, tessellate, bare except for scattered hairs at base and sides.. 12

    Abdominal fasciae absent, incomplete, or formed of somewhat sparse, partly erect hairs.. 13

12. Wings clouded at tips; dorsulum tessellate................................*nubecula* Sm.

    Wings not clouded at tips; dorsulum polished...........................*canadensis* D.T.

13. Distance from inner margin of eye to inner margin of facial fovea, along a transverse line through centres of antennal sockets, equal to or greater than one-half distance from inner margin of eye to outer edge of antennal socket............... 17

    Distance from inner margin of eye to inner margin of facial fovea less than one-half distance from inner margin of eye to edge of antennal socket.................... 14

14. Process of labrum truncate at apex.. 15

    Process of labrum rounded at apex... 16

15. Clypeus slightly produced, sparsely punctate except at base and lateral margins, with a distinct shining impunctate space; facial foveae nearly uniform in width; stigma and veins medium brown, second submarginal cell with sides subequal *novaeangliae* Vier.

    Clypeus convex, not produced, distinctly and somewhat closely punctate, roughened between punctures, no median impunctate space; facial foveae distinctly widened toward vertex; stigma and veins very dark brown, second submarginal cell distinctly longer than high, oblique.................................*algida* Sm.

16. Process of labrum arcuate, stigma and veins light brown; first recurrent vein received by second submarginal cell before its middle; clypeus sparsely punctate *vagans* Ckll.

    Process of labrum sub-triangular, rounded at apex, length equal to about one-half width of base; stigma and veins honey-yellow; first recurrent vein received by second submarginal cell beyond its middle; clypeus convex, closely punctate at sides, more sparsely toward middle with an irregular impunctate space.*salictaria* Robt.

17. *Process of labrum emarginate*; clypeus strongly punctate, roughened between punctures, no median impunctate space; abdomen thinly subfasciate; first tergum impunctate..***lata*** Vier.
Process of labrum not emarginate.. 18
18. Greater part of clypeus impunctate, polished, shining; process of labrum rounded-truncate; *dorsulum shining, first abdominal tergum* impunctate......***bipunctata*** Cress.
Greater part of clypeus punctate or roughened, at most with a median impunctate space.. 19
19. Second abdominal tergum impressed posteriorly about one-half its length; first tergum finely but distinctly and rather closely punctate; clypeus with a narrow *shining impunctate space, remainder strongly and regularly punctate; first* recurrent vein not received beyond middle of second submarginal cell..***wheeleri*** Graen.
Second tergum impressed distinctly less than one-half its length; first recurrent vein received beyond middle of second submarginal cell........................... 20
20. Clypeus coarsely and regularly punctate, strongly roughened between punctures, *with a very narrow indistinct median space; mesopleura densely clothed with* long gray hair; process of clypeus long, finger-shaped.....................***placida*** Sm.
Process of clypeus broad, truncate.. 21
21. Process of clypeus rounded truncate; facial foveae not distinctly widened toward vertex; clypeus very sparsely and irregularly punctate with a wide impunctate *space, slightly roughened and dull; first abdominal tergum shining, finely punc*tate; terga broadly testaceous at apex, remainder often reddish; stigma and veins light brown to yellow..***robertsoni*** D.T.
Process of clypeus squarely truncate; facial foveae distinctly widened above; clypeus polished; first tergum sparsely and obscurely punctate, roughened *between punctures; terga narrowly testaceous at apex; stigma and veins dark* brown.. 22
22. Clypeus produced, scutellum with two large polished areas, separated by a narrow line of punctures, these areas very sparsely punctate; hairs of tibial scopa rather short..***carolina*** Vier.
Clypeus *not produced; scutellum usually roughened all over*, or at least finely tessellate; hairs of scopa rather long, golden brown; terga with apical fasciae of gray, sub-erect hairs; first and second usually clothed with long hairs, others with short sparse pubescence; clypeus strongly and regularly punctate except for a narrow median space, somewhat T-shaped; mesonotum with punctures *rather distinct, spaces between punctures tessellate*..................***thaspii*** Graen.
23. Apical part of second tergum impressed distinctly less than half of its length........ 24
Apical part of second tergum impressed half or more than half of its length........ 25
24. Terga with usually complete apical fasciae; clypeus very closely and regularly punctate, pubescence mostly light gray..................................***kalmiae*** n.sp.
Terga without distinct apical fasciae, at most a sparse fringe of hairs; clypeus coarsely and rather sparsely punctate; pubescence mostly dull fulvous..***crataegi*** Robt.
25. Hind tibiae and tarsi stramineous to reddish; second tergum impressed about one-half; segments usually with partial fasciae of creamy appressed hairs at sides; punctures on clypeus very close and regular.....................***miranda*** Sm.
Hind tibiae and tarsi black or brownish; clypeal punctures somewhat irregular..... 26
26. Second tergum impressed three-quarters or more of its length..................... 27
Second tergum impressed distinctly less than three-quarters of its length........... 28
27. Space between fovea and margin of eye distinctly wider than fovea at level of antennae..***rugosa*** Robt.
Space between fovea and margin of eye not as wide as fovea at level of antennae ***grandior*** Ckll.
28. Space between fovea and margin of eye wider than fovea at level of antennae; abdomen usually with incomplete apical fasciae......................***ceanothi*** Vier.
Space between fovea and margin of eye distinctly narrower than lower part of fovea.. 29
29. Second tergum impressed one-half distance from base to apex; terga usually fasciate..***forbesi*** Robt.
Second tergum impressed slightly more than one-half distance from base to apex..***mariae*** var. ***concolor*** Robt.

Males

1. Facial line longer than transfacial line; clypeus shining, strongly but sparsely punctate, with an irregular yellow mark; pubescence of body light......***bradleyi*** Vier.
Facial line shorter than, or at most equal to, transfacial line........................ 2
2. *Cheek bordered posteriorly by a distinct keel or ridge, extending up toward the* posterior part of eye.. 7
Cheek rounded or toothed, but not keeled.... 3

3. Cheek with a prominent tooth or projection on its posterior margin; no black hairs on head........ 4
   Cheek rounded, without a prominent tooth, at most with a rounded angle........ 11
4. Mandibles with a prominent tooth near base on lower side........ 5
   Mandibles without a tooth near base on lower side........ 6
5. Face and cheeks with long erect hairs, some dark brown, remainder rufous; thorax and first abdominal tergum with long erect reddish hairs; some dark hairs on other terga........ ***milwaukeensis*** Graen.
   Face and cheeks with shorter hairs; pubescence of first tergum paler, ochreous to whitish........ ***mandibularis*** Robt.
6. Clypeus slightly produced; process of eighth sternum without a shoulder, narrowed behind apex, then widened again to basal plate........ ***carolina*** Vier.
   Clypeus not produced, head broad; process of eighth sternum with a shoulder, abruptly narrowed from it to apex........ ***thaspii*** Graen.
7. Third joint of antennae at least twice as long as fourth; keel on cheek very prominent; area of cheek between it and eye subquadrate; pleura with a slight rounded projection, less marked than in female of same species........ ***persimulata*** Vier.
   Third joint less than twice as long as fourth........ 8
8. Abdominal terga with more or less complete apical fasciae of appressed pubescence; head broad; area between margin of eye and keel much wider than eye in lateral view........ 9
   Terga with at most a very thin band of sparse sub-erect hairs; clypeus slightly produced; area between eye and keel scarcely wider than eye........ ***carolina*** Vier.
9. Fore wings clouded at apex........ ***nubecula*** Sm.
   Fore wings not so clouded........ 10
10. Apical fasciae closely appressed, remainder of tergum practically bare; mesonotum and scutellum shining; posterior edges of terga broadly testaceous........ ***canadensis*** D.T.
   Mesonotum and scutellum somewhat dull; apical fasciae of long, sub-erect, ochreous hairs; remainder of terga more sparsely covered with similar hairs........ ***hirticincta*** Prov.
11. Dorsal space of propodeum rugose, bounded posteriorly by a distinct transverse ridge, giving it a truncate appearance; terga with clean-cut punctures, shining between........ 24
   Dorsal space of propodeum rugulose, granulose, or nearly smooth, not truncate but produced on to posterior face of propodeum and ending in a sharp point, thus forming a triangular enclosure; terga usually roughened, finely, sparsely or not at all punctate (except *A. wilkella*)........ 12
12. Clypeus yellow........ 13
   Clypeus without yellow marks........ 14
13. Cheek only slightly wider than eye in lateral view; abdomen finely and closely punctate; posterior margins of terga broadly testaceous; legs and abdomen usually dull reddish........ ***robertsonii*** D.T.
   Cheek nearly twice as wide as eye in lateral view; abdomen tessellate, very sparsely punctate; posterior margins of terga narrowly testaceous; abdomen black; legs black to dull reddish brown........ ***bipunctata*** Cress.
14. Face or cheeks with some black or dark brown hairs........ 15
   Face and cheeks without dark hairs........ 18
15. Clypeus with pubescence mostly black, closely and strongly punctate; process of labrum sharply truncate; antennal joints three and four sub-equal; process of eighth sternum evenly rounded at apex........ ***algida*** Sm.
   Clypeus with pubescence mostly pale; apex of eighth sternal process truncate or slightly notched........ 16
16. Antennal joint three nearly twice as long as four; sides of propodeum with a large tuft of long black hair........ ***frigida*** Sm.
   Antennal joint three distinctly less than twice as long as four; sides of propodeum with pale hair only........ 17
17. Cheeks and angle between clypeus and eye with a predominance of black hair; joint three of antenna at most equal to joint four; process of eighth sternum with a dorsal and a ventral projection near apex........ ***carlini*** Ckll.
   Black hairs of head short and confined to a narrow strip between antenna and eye; joint three of antenna longer than four; process of eighth sternum simple ***regularis*** Mall.
18. Joint three of antenna almost twice as long as four; antennae reddish brown beneath; posterior borders of terga broadly testaceous; terga shining........ 19
   Joint three of antenna not conspicuously longer than four, sometimes shorter........ 20

19. Abdomen long, narrow, slightly reddish; process of eighth sternum long, narrow, tapering, almost acute at apex. . . . . *salictaria* Robt.
Abdomen rather broad; process of eighth sternum slightly widened toward apex, tip rounded; a slight ventral process or geniculation about two-thirds of distance from basal plate to apex. . . . . *placida* Sm.

20. *Larger, species 10 mm. or more in length; face and thorax normally covered with long, dense, erect ochreous hair; abdomen distinctly but finely punctate. 22*
Smaller, length 8 mm. or less; face and thorax normally covered with rather sparse whitish or slightly yellowish hair, thickest on clypeus; abdomen roughened, punctures very fine, nearly obscured by roughening. 21

21. Clypeus with large shallow punctures, partly obscured by roughening between them; enclosure of propodeum strongly rugulose at base; process of eighth sternum widened at apex, pilosity on ventral surface of process extending back to basal plate; no ventral production or geniculation, first abdominal tergum distinctly punctate. . . . . *wheeleri* Graen.
Clypeus with deep large punctures, shining between; enclosure of propodeum finely granular at base; process of eighth sternum with a prominent ventral geniculation, pilosity of process not extending more than half distance from apex to basal plate; abdomen usually with a slight greenish reflection. . . . . *lata* Vier.

22. Abdomen strongly and closely punctate, dull between punctures, with definite apical fasciae, usually complete on segments three and four. . . . . *wilkella* (Kby.) Ill.
Abdomen finely punctate, shining, no apical fasciae present. 23

23. Antennal joint three usually shorter than four; process of eighth sternum with its sides nearly parallel for the apical third when viewed from above. . . . . *vicina* Sm.
Antennal joint three equal to or longer than four; process of eighth sternum tapering toward apex from basal plate. . . . . *regularis* Mall.

24. Lateral angles of sixth abdominal sternum reflexed. . . . . *crataegi* Robt.
Lateral angles of sixth abdominal sternum not reflexed. 25

25. Antennae smooth and shining, joint three one-half as long as joint four; abdomen with incomplete apical fasciae. . . . . *forbesi* Robt.
Antennae dull, joint three more than one-half as long as joint four. 26

26. Posterior tarsi and usually tibiae bright reddish testaceous; abdomen with distinct but interrupted apical fasciae. 27
Posterior tibiae blackish or brown, tarsi dull brownish. 29

27. Rugae of enclosed dorsal space of propodeum nearly straight; process of eighth sternum narrow, apex rounded. . . . . *miranda* Sm.
Rugae of enclosed space coarse and irregular; process of eighth sternum truncate or slightly notched at apex. 28

28. Fourth antennal joint nearly twice as long as third; process of eighth sternum abruptly constricted about two-thirds of the distance from basal plate to apex, then gradually widened toward apex; apex rounded truncate. . . . . *ceanothi* Vier.
Fourth antennal joint only slightly longer than third, process of eighth sternum without a constriction in its process, abruptly widened at apex; apex slightly notched or bilobed. . . . . *grandior* Ckll.

29. *Dorsal space of propodeum coarsely rugose; clypeus evenly and closely punctate. 30*
Dorsal space of propodeum less strongly rugose; clypeus with a narrow and indistinct impunctate median space. . . . . *mariae* var. *concolor* Robt.

30. Dorsal space sharply separated from remainder of propodeum, very narrow at posterior edge; clypeus slightly produced; abdominal fasciae distinct, interrupted on some segments; pubescence of thorax pale ochreous. . . . . *rugosa* Robt.
Dorsal space very irregularly rugose, almost reticulate, broad at posterior edge, scarcely separated from remainder of propodeum, which is strongly wrinkled; clypeus short and broad; abdomen with fasciae indistinct or wanting; pubescence white or nearly so. . . . . *kalmiae* n.sp.

A. kalmiae n.sp.

Female

Black; head rather broad; clypeus closely and regularly punctate, with short, sparse, gray or slightly yellowish pubescence; process of labrum truncate or slightly emarginate, shining, much narrower at apex than at base; mandibles reddish brown almost to base, broad at tips, not crossing when closed; antennæ short, light brownish beneath, darker on top, joint 3 about

equal to 4 + 5; facial foveae broad, extending below antennal line, pubescence short, light brownish gray; cheeks shining, finely punctate, with light, sparse, appressed pubescence, longer near base of mandible, rounded, and not much wider than eye in lateral view.

Mesonotum closely punctate at sides, more sparsely so toward centre, tessellate between punctures, pubescence short, sparse, slightly brownish; pleurae rough, somewhat densely clothed with gray hair; scutellum coarsely and sparsely punctate except at posterior border where punctures are close; polished between punctures; metanotum rough, clothed with long hairs; enclosure of propodeum irregularly and closely rugose, truncate posteriorly; surrounding area also roughly rugulose or reticulate, lateral fringe light brownish; wings light brownish, veins and stigma dark testaceous, second submarginal cell narrow, receiving first recurrent vein beyond middle; legs dark brown at base, becoming reddish brown distally, tarsi yellowish, scopa golden yellow, flocculus lighter.

Abdomen shining, terga with narrow brownish posterior borders, distinctly and closely punctate, shining between punctures; pubescence light, short, apical fasciae usually complete on second, third, and fourth terga, but neither dense nor closely appressed, grayish in color; second tergum impressed less than one-third of its length; anal fimbria light brown. Length, 10-11 mm.

Male

Black; head broad; clypeus strongly and very closely punctate, sparsely pubescent; process of labrum broad, slightly emarginate; mandibles stout, reddish at tip; antennae rather stout, brownish, lighter toward tip, third and fifth joints subequal, fourth slightly shorter; cheeks rounded, slightly wider than eye, shining, finely punctate, hairs sparse, grayish.

Mesonotum, not very closely punctate, pubescence short, sparse except at sides, grayish; scutellum shining, with large sparse punctures; metanotum rough; pleura sparsely pubescent, coarsely wrinkled; propodeum much like female, hairs lighter; wings slightly darker than female, stigma reddish; legs dark, tarsi reddish testaceous, pubescence short, pale, yellowish on tarsi.

Abdomen comparatively short and broad, shining, terga often with light brownish borders, distinctly and somewhat closely punctate; hairs sparse, fasciae incomplete, or absent on some segments; second tergum impressed about one-third of its length; sterna with distinct apical fringes of rather long hairs; process of eighth sternum short, wide, abruptly widened at apex. Length 8-9 mm.

This species is very close to *A. daeckei* Vier., especially in the female characters. Miss Sandhouse has kindly compared the females of these species for the writer and has noted the following distinguishing characters: "Punctures of mesonotum finer and more widely separated in *kalmiae;* carinae on enclosure of propodeum in *daeckei* nearly longitudinal instead of irregular in direction; sides of propodeum below the row of long curled hairs shining, almost devoid of punctures or hairs; abdominal tergites with the punctures

of the impressed part more evident in ***kalmiae***, hair bands distinctly wider, abdominal terga of *daeckei* appear duller and browner; anal fimbria of ***daeckei*** *pale, hardly yellowish, antennal joint 3 equals one and a half times 4 and 5."* The writer has examined the male of *daeckei* and finds that it is smaller; inclining to brownish on abdomen; pubescence of a yellowish tinge; antennae light brown, propodeum with nearly longitudinal rugae much more regular than in ***kalmiae;*** veins and stigma dark ferruginous; process of eighth sternum rather slender, not conspicuously dilated at apex.

Types. Male and female, Kings County, Nova Scotia, Canada, July 9, 1931, on *Kalmia angustifolia* L.

Paratypes. One additional male of same date, two more July 13, 1932; 14 females June 24, 1931; 3 females July 9, 1931; 6 females July 13, 1932, collected by C. E. Atwood; one male and 5 females July 9, 1931, collected by Dr. W. H. Brittain; all on *Kalmia* and from Kings County, N.S. Types in the National Collection, Ottawa. Other specimens exchanged not in type series, including one male taken on *Ledum*, Kings County, June 24, 1932.

A. ceanothi Vier.

A. ceanothi Viereck, Trans. Am. Ent. Soc. 43 : 404; 1917.

Female

Black; clypeus shining, coarsely punctate, with punctures more numerous toward sides and base, very sparse toward apex, hairs dull fulvous; process of labrum squarely truncate; mandibles red at base; antennae black, joint 3 longer than 4 but not equal to 4 + 5; facial foveae broad above, abruptly narrowed from just above antennae to a narrow furrow with some long hairs in it as well as the usual appressed pubescence, and separated from eye margin by a space about equal to this narrowed part of fovea in width; cheeks rounded, narrow, finely and somewhat closely punctate, with pale fulvous hairs.

Mesonotum and scutellum coarsely punctate, punctures often adjoining along margins, with short, erect, fulvous pubescence; pleura very rough, with longer and paler hairs; enclosure of propodeum short, truncate behind, rugose, with about 20 distinct rugae, short at sides; sides of propodeum with pale fulvous hairs, rather long, surface strongly, irregularly rugose; wings faintly tinged with brown, veins and stigma dark brown, tegulae ferruginous; legs black or dark brown, tarsi reddish, flocculus ochreous, scopa and pubescence of legs in general pale fulvous.

Abdomen shining, densely punctate, second tergum impressed about two-thirds of its length, dense fasciae of whitish or rich creamy hairs on apical margins of terga, sparse fulvous hairs on basal parts of terga; anal fimbria reddish brown. Length 9-10 mm.

Male

Black; clypeus densely pubescent, closely and strongly punctate, with a suggestion of narrow median impunctate space, punctures often confluent;

process of labrum truncate; mandibles reddish at tip, black at base, apex bluntly rounded; antennae black or slightly brownish, rather stout, fourth joint almost twice as long as third and about equal to fifth; cheeks rounded, densely pubescent.

Mesonotum coarsely and regularly, not very closely punctate, tessellate between punctures, covered with erect hairs somewhat more dull than those of head; scutellum more coarsely and sparsely punctate, shining anteriorly; metanotum dull, strongly roughened, with obscure punctures; pleura densely pubescent, very closely punctate; enclosure of propodeum distinct, rather long, coarsely rugose; sides of propodeum coarsely wrinkled or reticulate, densely pubescent; wings brownish, veins dark testaceous or brown, stigma reddish; first recurrent vein received beyond middle of second submarginal cell; tegulae dark testaceous; legs dark reddish-brown near base, tarsi and posterior tibiae reddish stramineous, the latter with a dark spot, other tibiae sometimes partly yellowish; pubescence short, light.

Abdomen black, shining, terga with brownish apices, second tergum faintly impressed about one-third of its length; first and second terga finely but distinctly punctate, others more obscurely so; hairs very short and sparse, incomplete apical fasciae at sides; anal fimbria pale yellowish; process of eighth sternum sharply constricted near apex, then abruptly widened again at tip; basal plate of sternum very wide. Length about 8 mm.

The male which Viereck has described under this name is incorrectly associated with the female. The male described above was taken *in copula* with a female which agrees with Viereck's type. Viereck's male (paratype) differs from the above in the following details: clypeus with finer punctures, more sparse toward centre; pubescence shorter and more sparse; mandibles broad, pre-apical tooth small; antennae very slender, especially at base, brownish or reddish; scutellum and metanotum more closely punctate, shining; propodeum with short sparse hairs; tarsi ferruginous; abdomen slender, first tergum almost impunctate, highly polished; eighth sternum without a constriction near apex of process, but gradually widening from near middle of process to a rounded apex.

This will leave Viereck's male without a name but it is better not to propose a new one until someone has had an opportunity of associating it with the proper female, which, of course, may be already named.

Male, *in copula* with typical female, Kings County, Nova Scotia, Canada, May 18, 1931, on the cultivated plum (*Prunus*); 9 other males and 11 females, Kings, Digby, and Lunenburg counties (May 8 to July 3). Rather common on apple and plum.

The Genital Armature of *Andrena* and *Halictus*

The following drawings are an attempt to show some of the characters of the eighth and ninth abdominal sterna of males of the two genera under consideration. The writer's attention was drawn to these structures by a paper by Morice (4). The characters appear to be constant and of consider-

able taxonomic value, especially among the species related to *A. rugosa* Rob., where the males are much alike externally. Among the *Halicti* the characters are not so satisfactory but are still of value.

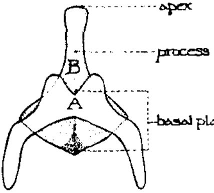

FIG 1. *Eighth ventral segment.*

Morice was somewhat mistaken in his interpretation of the structures which he illustrated. He considered that the "ventral valve (valvula ventralis)" was the eighth sternum, neglecting the "transferred segment" (propodeum), which would make the "ventral valve" the true ninth sternum, and the "stipes", bearing the various clasping organs, the tenth, whereas it is well known that the parameres of insects are found on the true ninth sternum. An examination of the ventral segments of *Andrena* will show a condition somewhat like that given by Fig. 1, which shows the eighth sternum in ventral view.

Eighth sternum of Andrena

The true eighth sternum is secondarily divided and the posterior and dorsal part, stippled and labelled "B" in the diagram, forms the "ventral valve". The anterior and ventral part of the eighth sternum has a posterior notch, into which the process of the "ventral valve" fits. This portion of the segment has been neglected in the drawings which follow.

The condition which obtains in the true ninth segment (Morice's "stipites") is shown in Fig. 2, which gives a dorsal view.

Ninth sternum of Andrena

The outer claspers have a common base, a dorsal "lobe", and a posterior and somewhat ventral "process", and enclose a second pair of claspers, which are the parameres. The aedeagus lies below these and is not visible in the dorsal view. The base and the parameres are neglected in the drawings.

The condition in *Halictus* is quite similar, but the eighth sternum is less apparently divided, and the processes of the stipites have other processes and brushes posteriorly and ventrally.

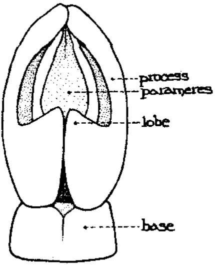

FIG. 2. *Ninth ventral segment.*

The drawings were made from mounts prepared by boiling the parts in potash solution and then mounting them. The structures of the *Andrenae* and the stipites of the *Halicti* were mounted in glycerine and small supports put under the cover glass to prevent crushing; the eighth sterna of the *Halicti* were stained and mounted in balsam. In general, the ventral view of the

eighth and the dorsal view of the ninth sterna are given, but in some cases dorsal or lateral views of the apices of the eighth sternal processes are also shown.

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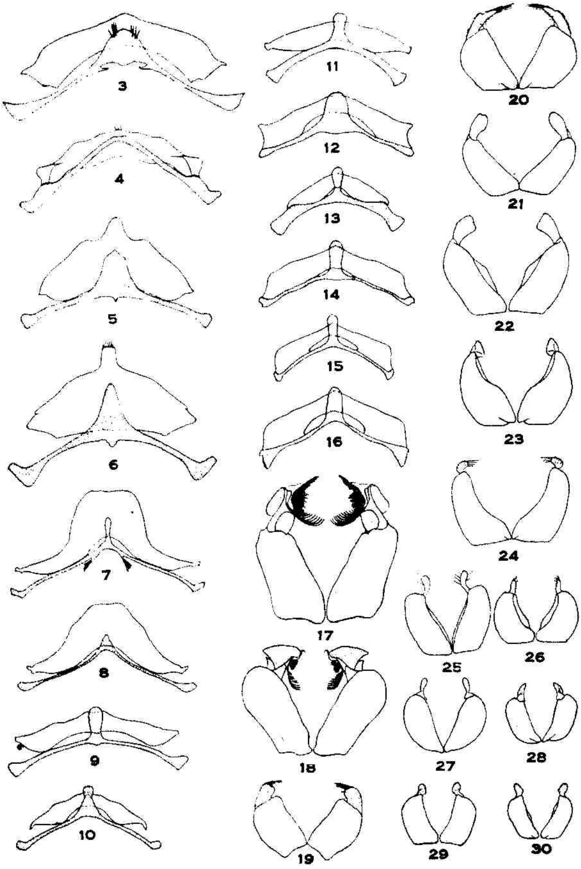
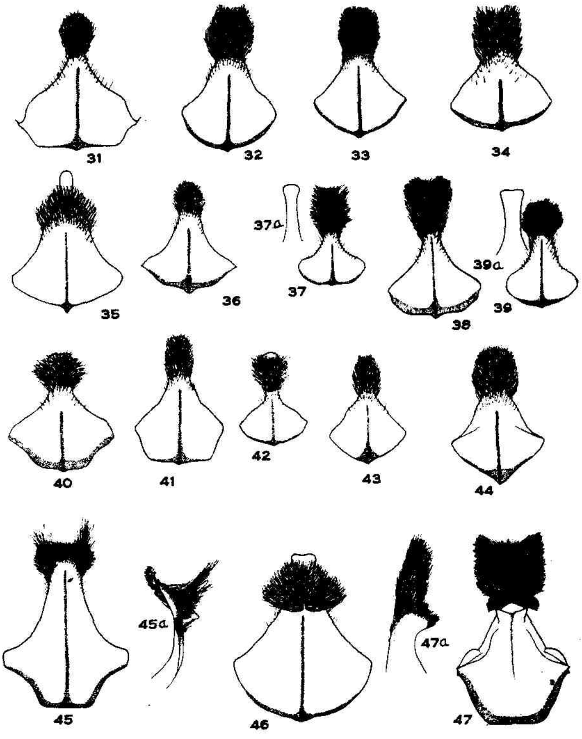
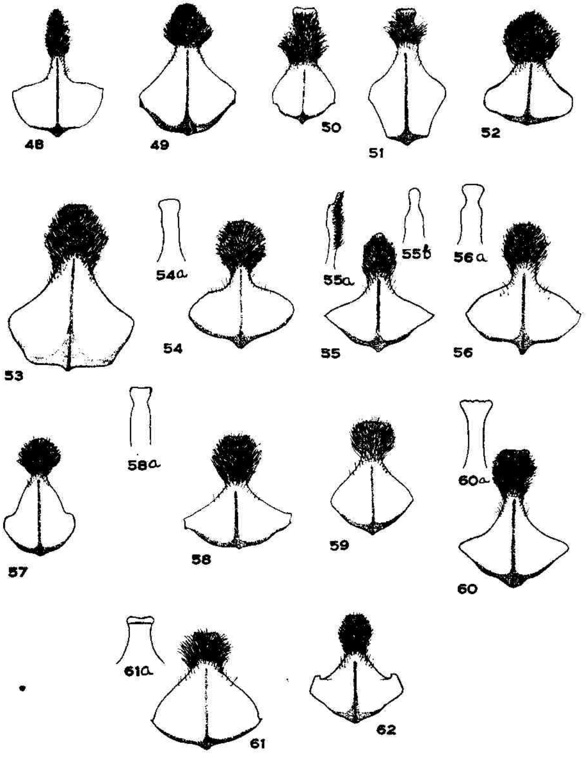


FIGS. 3–16. *Eighth ventral segments of male Halictus spp.* (×23) 3. *Halictus rubicundus* (*Christ.*) *Kby.* 4. *H. provancheri D.T.* 5. *H. athabascensis Sandh.* 6. *H. coriaceus Sm.* 7. *H. zonulus Sm.* 8. *H. leucozonius* (*Schrank*) *LeP.* 9. *H. arcuatus Rob.* 10. *H. pectoralis Sm.* 11. *H. rufitarsus Zett.* 12. *H. consonus Sandh.* 13. *H. foxii Rob.* 14. *H. viridatus Lov.* 15. *H. pilosus Sm.* 16. *H. nymphaearum Rob.*

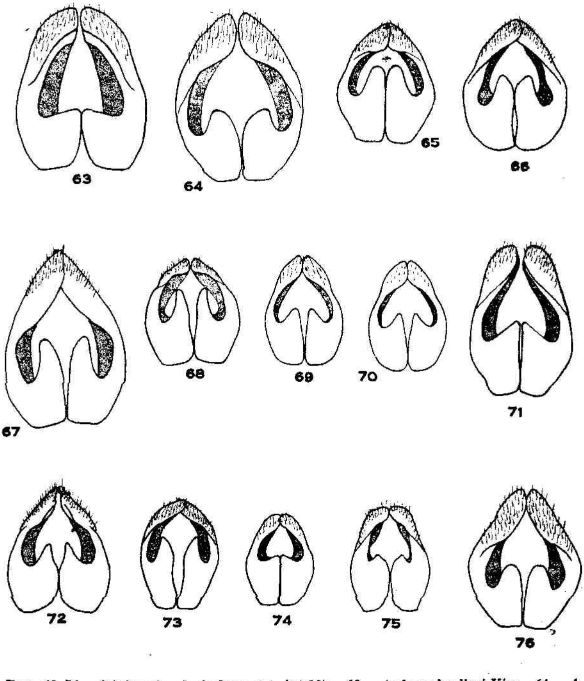
FIGS. 17–30. *Stipites of male Halictus spp.* (×23). 17. *Halictus rubicundus* (*Christ.*) *Kby.* 18. *H. provancheri D.T.* 19. *H. athabascensis Sandh.* 20. *H. coriaceus Sm.* 21. *H. zonulus Sm.* 22. *H. leucozonius* (*Schrank.*) *LeP.* 23. *H. arcuatus Rob.* 24. *H. consonus Sandh.* 25. *H. rufitarsus Zett.* 26. *H. pectoralis Sm.* 27. *H. nymphaearum Rob.* 28. *H. pilosus Sm.* 29. *H. viridatus Lov.* 30. *H. foxii Rob.*



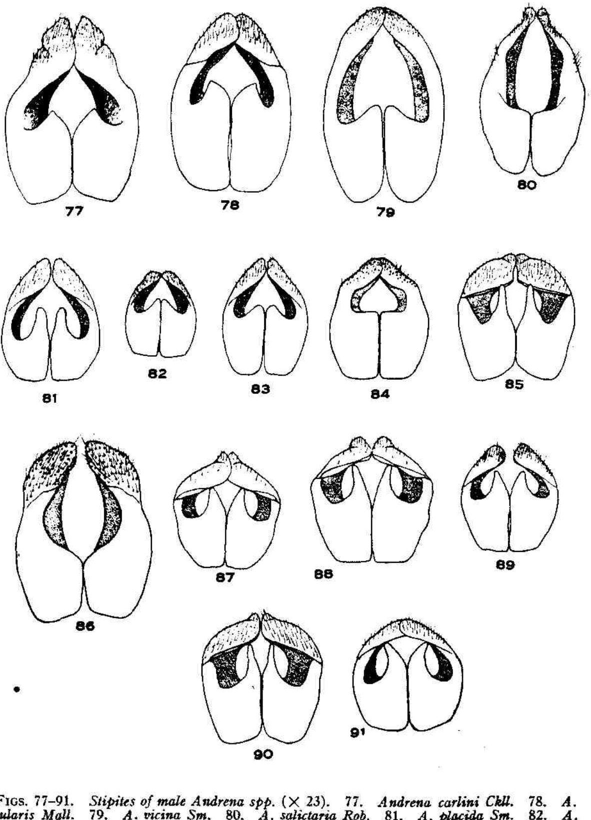
FIGS. 31–47a. *Part of eighth ventral segment of male Andrena spp.* (×23). 31. *Andrena bradleyi Vier.* 32. *A. milwaukeensis Graen.* 33. *A. mandibularis Rob.* 34. *A. carolina Vier.* 35. *A. thaspii Graen.* 36. *A. persimulata Vier.* 37. *A. nubecula Sm.* 37a. *A. nubecula (dorsal).* 38. *A. hirticincta Prov.* 39. *A. canadensis D.T.* 39a. *A. canadensis (dorsal).* 40. *A. robertsonii D.T.* 41. *A. bipunctata Cress.* 42. *A. algida Sm.* 43. *A. vagans Ckll.* 44. *A. frigida Sm.* 45. *A. carlini Ckll.* 45a. *A. carlini (lateral).* 46. *A. regularis Mall.* 47. *A. vicina Sm.* 47a. *A. vicina (lateral).*



FIGS. 48–62. *Part of eighth ventral segment of male Andrena spp.* (*cont'd.*) (× 23). 48. *Andrena salictaria Rob.* 49. *A. placida Sm.* 50. *A. wheeleri Graen.* 51. *A. lata Vier.* 52. *A. wilkella* (*Kby.*) *Ill.* 53. *A. crataegi Rob.* 54. *A. forbesi Rob.* 54a. *A. forbesi* (*dorsal*). 55. *A. miranda Sm.* 55a. *A. miranda* (*dorsal*). 55b. *A. miranda* (*lateral*). 56. *A. ceanothi Vier.* 56a. *A. ceanothi* (*dorsal*). 57. *A. sp.* (*incorrectly described by Viereck as the male of ceanothi*). 58. *A. rugosa Rob.* 58a. *A. rugosa* (*dorsal*). 59. *A. grandior Ckll.* 60. *A. mariae var. concolor Rob.* (?). 60a. *A. mariae var. concolor Rob.* (?) (*ventral*). 61. *A. kalmiae n.sp.* 61a. *A. kalmiae* (*dorsal*). 62. *A. daeckei Vier.*



FIGS. 63–76. *Stipites of male Andrena spp.* (× 23). 63. *Andrena bradleyi Vier.* 64. *A. milwaukeensis Graen.* 65. *A. mandibularis Rob.* 66. *A. carolina Vier.* 67. *A. thaspii Graen.* 68. *A. persimulata Vier.* 69. *A. nubecula Sm.* 70. *A. canadensis D.T.* 71. *A. hirticincta Prov.* 72. *A. robertsonii D.T.* 73. *A. bipunctata Cress.* 74. *A. algida Sm.* 75. *A. vagans Ckll.* 76. *A. frigida Sm.*



FIGS. 77–91. *Stipites of male Andrena spp.* (× 23). 77. *Andrena carlini Ckll.* 78. *A. regularis Mall.* 79. *A. vicina Sm.* 80. *A. salictaria Rob.* 81. *A. placida Sm.* 82. *A. wheeleri Graen.* 83. *A. lata Vier.* 84. *A. wilkella (Kby.) Ill.* 85. *A. forbesi Rob.* 86. *A. crataegi Rob.* 87. *A. miranda Sm.* 88. *A. ceanothi Vier.* 89. *A. grandior Ckll.* 90. *A. mariae var. concolor Rob.* (?). 91. *A. kalmiae n.sp.*

EXPERIMENTAL DRYING EQUIPMENT FOR ALIMENTARY PASTES[1]

By D. S. Binnington[2] and W. F. Geddes[3]

Abstract

An apparatus is described whereby the experimental drying of alimentary pastes may be conducted automatically under a pre-determined program of temperature, time and humidity relations.

The air employed for drying is continuously recirculated, dehumidification being obtained by by-passing an increasing proportion over refrigerating coils through the medium of an especially designed valve, operated electromagnetically and timed by an electric clock.

Complete control of the time-humidity relation is obtained by a regulated steam input from a water resistance boiler controlled by the action of the wet bulb recording pen in conjunction with a conducting strip placed on the recorder chart.

While specifically designed for drying alimentary pastes, the apparatus should be well adapted to other classes of drying studies and may also be employed to maintain constant conditions of temperature and humidity.

Introduction

The removal of excess water from various forms of alimentary pastes, such as macaroni and spaghetti, is generally conceded to represent the most critical stage of the entire manufacturing process. If the operation is unduly rapid, surface drying occurs, resulting in cracking, checking and curling, and if too slow, there is danger of chemical and bio-chemical changes, such as souring and molding, taking place.

The critical factor in the drying operation appears to be the rate of diffusion of water from the centre to the outside of the material, and the ideal drying condition, therefore, is one that will remove the surface moisture at a rate that exactly parallels this diffusion. Modern commercial practice approximates this condition by drying under a falling humidity gradient, which is obtained by: *A*, raising the temperature of the air used for the drying process; *B*, allowing a portion of the moisture laden air to vent to the atmosphere, replacing it by air at atmospheric humidity; *C*, a combination of *A* and *B*. The first method, *A*, is subject to the disadvantage of the comparatively high temperatures needed to obtain the desired reduction in humidity. The use of such temperatures is considered by the older school of macaroni makers to be very detrimental to the product. No definite evidence however appears to be available on this point. The chief criticism of methods *B* and *C* is that they are not accurately controllable, owing to variations in atmospheric humidity, and are therefore of doubtful value for experimental studies.

[1] *Manuscript received December 13, 1933.*
Contribution from the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada. Published as Paper No. 48 of the Associate Committee on Grain Research, National Research Council of Canada.

[2] *Research Assistant, Associate Committee on Grain Research.*

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The testing of durum wheats for quality obviously requires that the entire sequence of operations from the milling of semolina to the drying of the finished product be conducted under carefully controlled and reproducible conditions. This was the principal consideration in the design of the apparatus to be described. However, in view of the fact that so little information is available regarding the effects of varying conditions of time, temperature and rate of humidity fall, on the drying operation, it was deemed advisable to make suitable provision for investigating these different factors.

A preliminary survey indicated that no commercially built apparatus was available that would fulfil the necessary requirements, and that equipment in the few existing small-scale units was not capable of sufficiently accurate control for our purpose. It thus became necessary to design and construct a drying cabinet that would:—

(i) maintain a constant temperature throughout the range from room temperature to 120° F;

(ii) produce and maintain any desired relative humidity up to and including saturation within the entire temperature range, and independent of external humidity;

(iii) incorporate means for producing an automatically falling humidity gradient, and for varying this rate of fall within wide limits, but keeping the temperature constant at any desired value;

(iv) incorporate means for producing an automatically falling humidity gradient by slowly and progressively raising the temperature;

(v) incorporate means for circulating the "conditioned" air in a stream of uniform cross-sectional velocity over the material to be dried, with provision for varying the velocity if necessary;

(vi) provide suitable instruments for indicating and recording temperature and relative humidity.

The apparatus as finally developed operates as follows. Air is forced by a fan through a drying chamber containing the macaroni or spaghetti to be dried. This is suspended in the conventional manner over round wooden sticks which are supported on suitable carriers. The moisture laden air leaving the drying chamber may be divided into two streams by a valve, one passing through a section containing refrigerating coils, the other flowing through a by-pass. The air streams unite, mix, and pass to a chamber containing heating coils thermostatically controlled, before returning to the drying chamber. A closed circuit is thus maintained, the same air being continuously recirculated. By gradually and progressively opening the valve, an increasing proportion of the air is caused to flow over the cooling coils, moisture is frozen out, and a decrease in the relative humidity of the air returning to the cabinet is obtained. Thus, by varying the rate of motion of this valve, any desired humidity gradient may be produced, temperature being kept uniformly constant.

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Constructional Details

The general constructional details are fully illustrated in Fig. 1 together with the location of the accessory heating, humidifying and dehumidifying apparatus.

Framework and Cabinet

The framework was built of 3 by 3 in. stock in three sections, afterwards bolted together, using strips of felt between the adjacent faces to act as gaskets. The central section contains the drying chamber proper which is built from 1 by 4 in. tongued and grooved fir. This chamber was constructed in such a manner that the carriers when introduced, occupy the entire cross-sectional area, compelling the air stream to flow through the material being dried. Three rabbeted doors are fitted, equipped with double glass for thermal insulation. These are provided with rubber refrigerator door insulation and standard refrigerator hinges and catches in order to secure a reasonably air-tight fit. The entire interior surface is moisture proofed with two coats of hot raw linseed oil, and three coats of high grade spar varnish. Two small inspection windows, 6 by 8 in., are placed in the top of the drying section. The end sections containing the inlet and outlet cones are completely panelled with three-ply veneer, with the exception of the left end, which is fitted with a slate panel, serving as an instrument board.

Seven galvanized iron louvres, 4 by 23½ in., are fitted at each end of the drying chamber, permanently attached to ¼ in. brass rods passing through the front and back walls of the cabinet. At the rear, a steel bearing plate is provided, attached to the outer wall of the drying chamber. Tension to maintain the louvres in place is obtained with short coil springs, held in place by washers and split pins. The front bearing plate is of brass, and is attached to a removable panel, rabbeted and provided with felt gaskets. The entire louvre assembly may be removed together with this panel by unscrewing and detaching the tension springs at the rear. The forward ends of the brass rods are provided with knurled knobs to which are attached short arms fitted with steel locating pins. These pins may be locked in any one of a number of small holes drilled in the brass plate, by simply pulling the rod forward against the spring tension, rotating to the desired position and releasing. In this way adjustment of the louvres and consequent uniformity of air flow may be secured and maintained without opening the doors of the drying cabinet.

The Fan

This is a "Series 30" Size No. 1, Canadian Sirocco, equipped with a ⅛ h.p. motor. After being placed in service, difficulty was experienced owing to leakage and venting of air from the apparatus, which was finally traced in part to a constructional feature of this fan. With small sized fans of this type, a special bearing for the rotor is dispensed with, the entire load being carried by the motor bearings. Presumably owing to this fact, a comparatively large opening is made in the side of the fan housing allowing

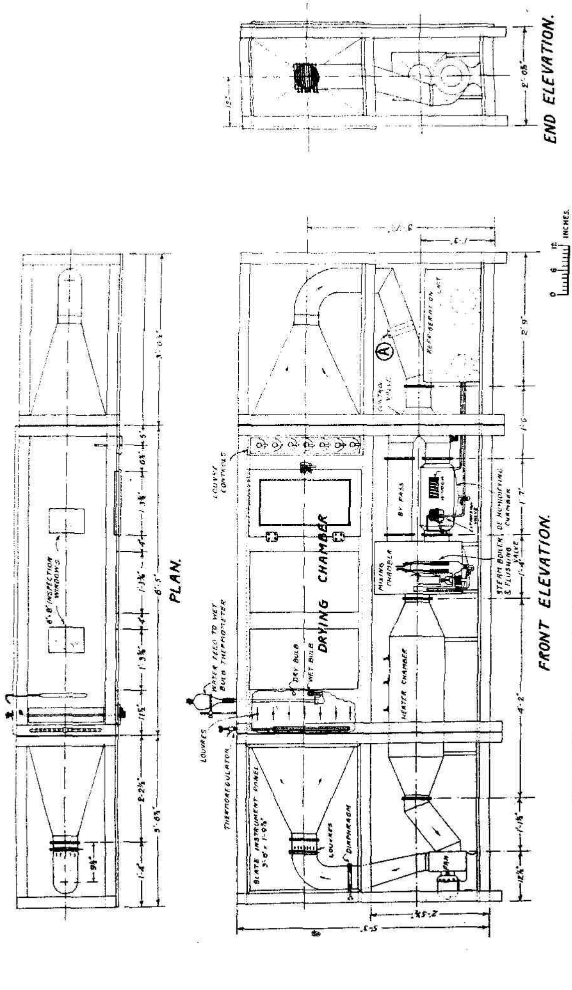

FIG. 1. *General working drawing showing main constructional features and location of accessory apparatus.*

$\frac{1}{4}$ to $\frac{3}{8}$ in. clearance around the fan hub. At high speeds, and particularly in a closed circuit, steady leakage takes place through this opening. Heavy felt gaskets were tried in an attempt to seal this opening, but lasted only a short time, and final solution of the difficulty was accomplished only by entirely enclosing the space between the end of the motor casing and the side plate of the fan. This was done by soldering a short sheet metal cylinder directly onto the end of the motor and luting the other end of the cylinder to the fan housing with battery pitch. Suitably closed apertures were provided for oiling the motor bearing, and attaching the fan to the motor shaft. The regular ventilation opening in the end of the motor was closed with a metal plate, and other openings drilled external to the enclosing cylinder. A metal slide accommodating brass diaphragm plates of various apertures is fitted between the fan and the inlet cone, thus making possible control of air velocity without altering the speed of the fan.

Sheet Metal Work

The various chambers and ducts of the apparatus are built from 26 to 28 gauge galvanized iron, 18 gauge being employed for the inlet and outlet cones.

In order to facilitate the assembly of the various parts, brass flanges cast from an especially prepared pattern are employed. These are mated in pairs, provided with locating marks, and soldered to the portions to be connected. Gaskets of $\frac{1}{16}$ in. sheet rubber are used to secure a tight joint. The entire assembly of auxiliary apparatus is made in this manner (working from left to right), with the exception of the final connection, (point "A") Fig. 1. This is made by butting the ends of the air ducts together, surrounding by a clamping band of heavy sheet metal, and sealing the edges with battery pitch. This material is used throughout for sealing minor openings, larger ones being stopped by a plastic mass of paper towelling pulped with a hot rosin soap solution.

Heating System

This consists of suitable elements placed in the air stream, and controlled by a sensitive thermoregulator.

The heating unit is made from 18 B and S gauge Chromel "A" wire threaded back and forth between two sheets of asbestos transite $\frac{1}{4}$ by $11\frac{1}{2}$ by $29\frac{1}{2}$ in., spaced $10\frac{1}{2}$ in. apart by $6\frac{1}{4}$ in. brass rods. The wire is introduced in uncoiled lengths of approximately 10 ft. and connected to bolts at each end; 28 of these units are joined in series of seven, giving four heaters of 3.8 amperes capacity each. This method of wiring, though tedious, gives a heating unit of large radiating surface and very low lag.

This heater is housed in a rectangular metal section, 30 by 12 by 12 in. (exclusive of the coned ends), and lined throughout with asbestos paper. The entire heating unit is inserted through the front, which is then closed by a flat metal plate bolted onto flanged edges covered with strips of sheet rubber. The four heaters are connected to individual switches, two being

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controlled by a *mercury-toluene* thermoregulator of the grid type, similar to that described by Wing (2), placed immediately behind the louvres at the inlet end of the drying chamber. An American Instrument Company supersensitive relay with a maximum capacity in excess of 1000 watts is employed. This relay is mounted on the control panel together with a pilot lamp. The upper (adjustable) contact of the thermoregulator projects through the top of the cabinet, so that final setting of temperature may be accomplished without opening the doors.

Mixing Chamber and Humidifier

The mixing chamber, 12 by 21 by 8 in., contains approximately 30 sq. ft. of 12 mesh bronze gauze, suspended in strips $\frac{1}{4}$ in. apart. Humidification is accomplished by means of a steam generator (Fig. 2), built of Pyrex glass, employing the principle of direct water resistance as a heating medium.

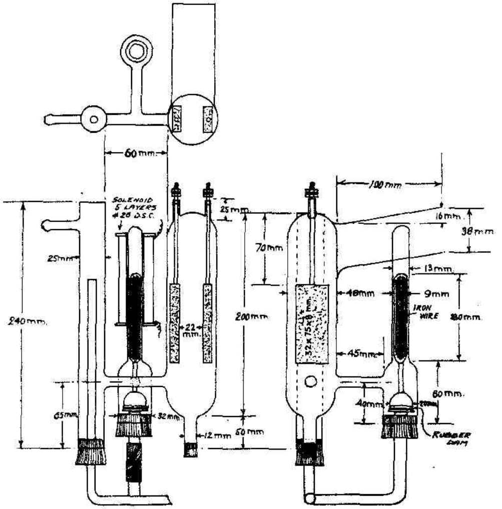

FIG. 2. *Details of steam generator and flushing device.*

Carbon plate electrodes of 3 sq. in. area each, threaded onto brass rods are used, a constant feed serving to supply tap water to the boiler. Owing to the small amount of water contained in the boiling chamber, steam is generated in approximately one minute and ceases the instant that either the water supply is exhausted or the current interrupted.

Continued operation of this type of boiler results in an accumulation of soluble salts which decrease the resistance appreciably. Uniformity of operating characteristics may be secured by draining manually at fairly frequent intervals, but for long runs automatic control is desirable. This is attained by a simple valve actuated by a solenoid, the rate of operation of which is controlled by an electric clock with suitably spaced contacts. A single contact of suitable width on the face, and a brush on the minute hand giving one flush of about 30 to 45 sec. duration per hour, is entirely satisfactory with the water supply available to the writers. The entire assembly of boiler, flushing valve and constant level is constructed from Pyrex glass, as a single unit, and is illustrated in Fig. 2.

The presence of the bronze mesh in the chamber assists in mixing the entering air streams. Baffles are provided over the inlets and exit in this chamber to minimize entrainment of water spray in the air stream.

Dehumidifying Equipment

Dehumidification of the circulating air is accomplished in a chamber containing 90 ft. of $\frac{3}{8}$ in. copper tubing wound in three concentric layers. Liquid sulphur dioxide is supplied to this coil from a $\frac{1}{3}$ h.p. Frigidaire Compressor unit (Model A 233), through a thermostatically controlled expansion valve. The methyl chloride thermostat bulb actuating this control is placed in close contact with the cooling coils. In addition, the valve is equipped with a manual control, which may be adjusted for any desired back pressure or vacuum that experience may dictate, a suitable vacuum and pressure gauge being provided. A trapped drain is placed in the bottom of the refrigerating section to remove condensed water as it accumulates.

Valve and Operating Mechanism

The valve is illustrated in Fig. 3. The body is built up from $\frac{3}{32}$ in. brass plate, the edges being butted together and soft soldered. The valve plate is cut from $\frac{1}{8}$ in. brass, and is attached to its shaft by small machine screws, and balanced by an external counterweight. If sufficient care is exercised in the construction of the casing and the fitting of the valve itself, not more than $\frac{1}{64}$ in. clearance at each side should be necessary for free working. Adjustable stops are provided at each end of the travel in order to prevent the valve from locking. These stops are set to $\frac{1}{32}$ in. opening.

The operating mechanism is shown in detail in Fig. 4. It consists of a ratchet (184 teeth) actuated by an electromagnet. The motion of the ratchet is communicated to the valve by a train of gears (5 to 1 reduction) through the medium of a flexible coupling. When current is supplied to the electromagnet the pawl is pulled forward into the next space of the ratchet. As soon as the current is interrupted, the pawl is returned to its original position by means of a tension spring, thereby causing the ratchet to move through the distance represented by a single tooth (approximately $\frac{1}{16}$ in.). The electromagnet is wound with four layers of 18 B and S gauge bell wire, and

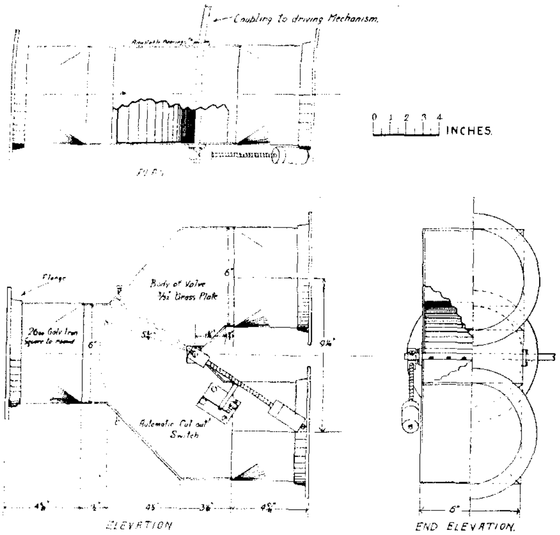

FIG. 3. *Details of by-pass valve.*

operates on a six volt battery at seven amperes. A switch operated by the valve counterweight is provided in order to disconnect the electromagnet when the valve reaches the end of its travel.

The timing of the impulse is obtained with an electric clock. This is provided with 24 narrow brass contacts set flush in an insulating ring of hard rubber, and spaced at $2\frac{1}{2}$ min. intervals. Each contact is wired to an individual switch, the circuit being completed by a very small brush of platinum foil attached to the end of the minute hand. A two-volt current is employed in this circuit to operate a relay, which controls the current to the electromagnet. With every switch in the circuit, the valve thus receiving an impulse every $2\frac{1}{2}$ min., approximately 5 hr. are required to effect complete opening. By cutting out the switches in suitable order the valve may be opened in 5, 10, 20, 30, 40, 60, or 120 hr. In addition, this order may be varied, thus shortening or extending the rate of opening at any desired stage of the drying operation.

Temperature and Humidity Recording Instruments

Changes in temperature and relative humidity are followed by means of a 24-hr. chart two-pen recording thermometer, one bulb of which records air

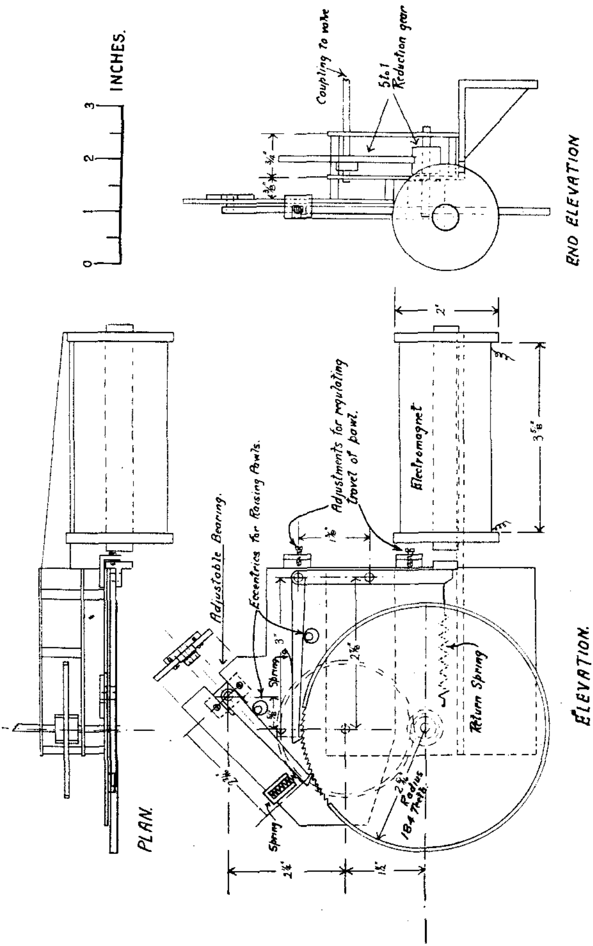

FIG. 4. Details of valve operating mechanism.

temperature, and the other, wet bulb temperature. The bulbs are placed at the inlet end of the cabinet, as indicated in Fig. 1. The lower (wet bulb) is wrapped in thin silk and fed with distilled water from four separate wicks dipping into cups attached to a common header crossing the cabinet from side to side, and provided with levelling adjustments at each end.

Wiring and Controls

The controls, switches, recording thermometer, relays and timing clock are located on the slate instrument panel previously mentioned. Wiring is carried to this point in No. 14 B.X. and divided into four circuits individually fused before connecting to the 110-220 volt line. The entire wiring diagram is illustrated in Fig. 5.

Humidity Gradient Control

Initial tests with the apparatus as described showed a rapid drop in relative humidity from saturation to about 82%. This drop is probably due to leakage of air from the system, and may be offset by means of an automatically regulated steam input from the humidifier.

Commercial control instruments are available for the production of predetermined time-temperature and time-humidity gradients. These instruments produce the required gradients by means of metal cams which actuate a compressed air system of valve control. Both fixed and variable cams may be obtained but the degree of adjustment in the latter type is confined to a limited portion of the time range. For experimental work, a large number of cams would be required and it seemed advisable to devise a method of control which would allow of the production of any desired humidity or temperature gradient without recourse to the cutting of special cams. Moreover, high pressure steam and compressed air were not available.

The method of regulation finally adopted is based on an apparatus recently developed by Warren (1) for producing a controlled temperature program in a muffle furnace. He employs conducting strips of aluminium foil cemented on to the chart of a potentiometric recorder, the strips being cut to conform to a definite temperature-time gradient, a stylus travelling between the strips which are separated by a distance of 0.16 mm. Resistances are so arranged that a falling temperature, resulting in the pen contacting with the lower strip, cuts out resistance, and a rising temperature adds resistance.

In applying this principle to humidity control, a sufficient current is supplied to the direct resistance boiler to maintain it slightly below the boiling point. As the humidity in the cabinet falls, the wet bulb pen contacts with a conducting strip on the chart, operating a relay which shorts out the resistance in the boiler circuit. Steam is thus generated instantaneously, stopping the moment the pen leaves the conducting surface. It is thus possible to make the wet bulb record follow any predetermined course, rising, falling or constant. In the case of a falling curve it is, of course, necessary to continuously remove water at a suitably regulated rate. This can be readily controlled by the valve and timing arrangement previously described.

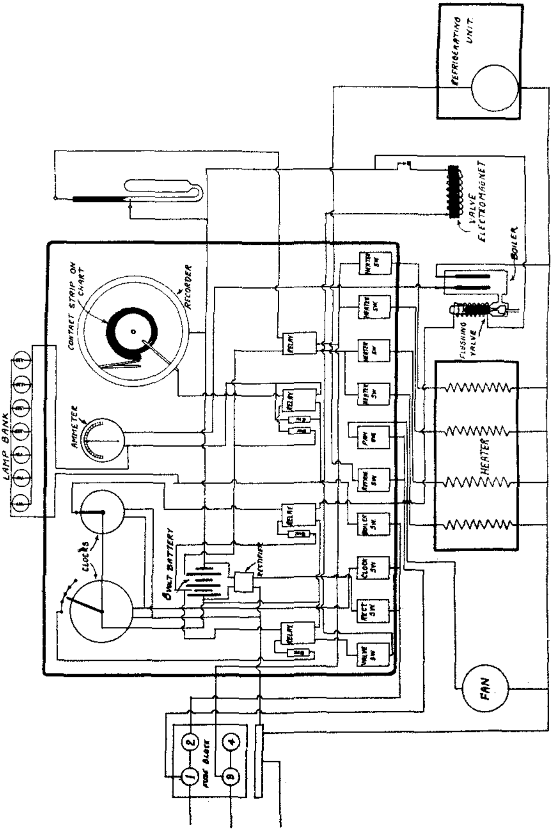

FIG. 5. *Wiring diagram.*

The principle of adding and withdrawing moisture simultaneously to maintain a desired relative humidity is analogous to that employed in maintaining a thermostat below room temperature by the combined use of heating and cooling devices.

Considerable difficulty was experienced in securing a satisfactory contact surface; aluminium foil as used by Warren (1) could not be cut and fitted with sufficient accuracy to a circular chart, gave poor electrical contact, and also obstructed the free travel of the pen. Satisfactory results were finally attained by two methods, the first being suited to the production of a chart required once only, the second for a permanent chart that may be used repeatedly.

Method I

Precipitated copper powder is mixed with a cellulose lacquer, painted on to the chart in a strip, the outer edge of which conforms to the required curve, dried, and burnished until a conducting surface is obtained. Some little experience is necessary to secure uniform results by this method and the charts deteriorate rather rapidly, generally losing their conducting power after a few days.

Method II

The required curve is plotted on a paper chart and a circular disk of celluloid superimposed. This disk is provided with a central hole of the same size as the chart, the two being clamped together on a metal plate by means of a central bolt or mandril. The conducting strip is then painted on to the celluloid as in Method I. When dry it is burnished, a conducting wire attached, and copper-plated in a cyanide bath, using a large circular sheet of the metal as an anode. With a $\frac{1}{2}$ in. separation approximately 30 min. with a current of 0.15 amp. is satisfactory. Too heavy a plate tends to cause the coating to peel. The copper powder is prepared by gradually adding zinc dust to copper sulphate solution, stirring vigorously until nearly decolorized, washing with dilute hydrochloric acid, followed by water until free from acid. The precipitated metal is then transferred to a Buchner funnel, washed with alcohol and ether and stored under c.p. amyl acetate until required.

A suitable cellulose lacquer may be prepared by dissolving celluloid clippings in a mixture comprised of equal volumes of amyl acetate, ethyl acetate and acetone and adding 1 to 2% of dibutyl phthalate as a plasticizer. The mixture of copper powder and lacquer is made in a small agate mortar and only as required, a reaction apparently taking place which causes a gradual thickening, thus impairing its working qualities and affecting the conductivity.

An adjustable brush contact is fitted to the case of the recorder, the wet bulb pen itself completing the circuit. Ink may be used in this pen without interfering with its function as a contact. A two volt current is employed

to actuate a standard "Pony" telegraph relay, fitted with heavy wiring and large silver contacts. This relay controls the resistance employed to regulate the idling current on the boiler, which consists of a bank of 100-watt lamps. A current of approximately 1.4 amperes is sufficient to maintain the temperature just below the boiling point.

This control functions very smoothly; no appreciable deviation of the wet bulb pen from the contact edge can be observed and any form of curve is accurately followed, the only limit to the steepness of falling gradient obtainable being imposed by the rapidity with which the refrigerator can remove moisture. This same method of control may be employed to produce a rising temperature-time gradient by placing the contact strip above the dry bulb pen, and connecting to the heater relay in place of the thermoregulator.

Operation

The operation of the whole assembly in drying a charge of macaroni or spaghetti is as follows. The required humidity-time curve is plotted and humidities read off and tabulated for each hourly interval. The desired dry bulb temperature is then selected and the hourly humidities calculated to the corresponding wet bulb temperatures. These temperatures are then plotted on a recorder chart (or charts) and a control chart prepared by either Method I or II. Air circulation is then started, the temperature raised to the desired value and the thermoregulator adjusted. When constancy of dry bulb temperature has been attained, steam is admitted until saturation is reached, the air flow checked with a small diaphragm and the charge introduced. The resistance on the boiler is then regulated to maintain approximate saturation and the charge allowed to come to equilibrium (about two hours). The control chart is then fitted, the refrigerator started, and the valve control set for appropriate time intervals; the diaphragm is removed and the boiler resistance readjusted. Drying now proceeds automatically according to the predetermined schedule. The only necessary attention needed is to change the control chart if the drying schedule exceeds 24 hr. and possibly to defrost the cooling coils towards the end of a long run.

Acknowledgments

The authors are indebted to the staff of the Engineering Department of the University of Manitoba for valuable suggestions and assistance in the construction of the apparatus. They are also indebted to Mr. Eric Able for the suggestion from which the valve control was finally developed.

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THE PRECIPITIN RING TEST APPLIED TO SOME USTILAGINACEAE[1]

BY E. C. BECK[2]

Abstract

The standard precipitin ring test was applied in an effort to differentiate the following smut fungi: (1) monosporidial cultures of *Sorosporium reilianum* (Kühn) McAlp., *Ustilago hordei* (Pers.) K. & S., *Ustilago levis* (K. & S.) Magn., *Ustilago zeae* (Beck.) Ung., and (2) mass cultures of *Ustilago hypodites* (Schlecht.) Fr., *Ustilago tritici* (Pers.) Rost., *Ustilago avenae* (Pers.) Jens., *Ustilago levis* (K. & S.) Magn., and *Ustilago zeae* (Beck.) Ung. The results obtained indicate that monosporidial strains could be satisfactorily differentiated. Although they were frequently mutually reactive in undiluted serum-antigen mixtures, their specificity was apparent in the persistence of the precipitin ring at greater dilutions with homologous than with heterologous mixtures. Mass cultures were not so satisfactorily differentiated and showed many cross reactions. The results indicate that closely related species and physiological forms of fungi are not easily differentiated since characters common to two or more of them apparently tend to mask their ultimate differences. The method, however, offers possibilities as regards the more intimate affinities among groups of doubtful species or genera.

Introduction

The present investigations were undertaken in an attempt to determine the usefulness of the precipitin ring test in the identification of fungi. The general limits of its usefulness were to be determined by its applicability to the various species of Basidiomycetes, the Ustilaginales and Uredinales, and if the results thus warranted, the method was to be extended to the differentiation of physiological forms within the species previously employed.

While this study was in progress Link and Wilcox (6, 7) reported on extensive investigations along the same general line. Since these have shown very satisfactorily the limitations of the method in differentiating various species and strains of the Fungi Imperfecti, it has been decided to discontinue the project, and to publish the preliminary results which have been obtained to date and which are in general accord with those obtained by Link and Wilcox.

Materials and Methods

The cultures of monosporidial origin from which extracts were obtained were procured through the courtesy of Dr. J. J. Christensen of Minnesota University, and are designated by the superscript letter C, namely *Ustilago zeae*^{C}, *Ustilago hordei*^{C}, *Ustilago levis*^{C} and *Sorosporium reilianum*^{C}. The culture of *Ustilago hypodites* was isolated from *Agropyron repens*, collected and identified by Dr. R. E. Fitzpatrick of this department. *Ustilago zeae* was isolated from corn collected in a nearby field. The remainder, namely *Ustilago avenae*, *Ustilago tritici* and *Ustilago levis* were isolated from materials provided by Professor J. E. Howitt, of the Ontario Agricultural College, Guelph.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ontario, Canada.
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In making the isolations from the mass cultures, monospory was not attempted, as it was thought that a comparison of bulk cultures with those of monosporidial origin might reveal some interesting differences. Spores were removed by means of sterile forceps from the inside of smut galls, and transferred to the following smut medium recommended and used by Stakman, Christensen *et al.* (9): potato extract, 1.8% agar, 1.0% dextrose, 1.0% sucrose and 1.0% malt extract. The medium was dispensed in eight-ounce medicine bottles and incubated at 26° C. for two days prior to inoculation.

After inoculation the cultures were incubated at 26° C. for two weeks, during which time a heavy mass of growth developed over the entire surface of the medium. This surface growth being in most cases cohesive, was easily removed by means of a stout lance-shaped needle. Those less cohesive were washed from the agar surface by means of 0.85% sodium chloride solution and removed to a sterile mortar and ground in fine, chemically clean sterilized quartz sand. To each inoculum was added 50 cc. of 0.85% sodium chloride solution. The resulting finely ground product was digested in the ice box for 18 hr. before being filtered under pressure through a layer of glass wool which was covered with filter paper in a Büchner filter. The filtrate was filtered through No. 4 Mandler bacteria-proof filters. The crystal clear extracts which resulted were dispensed in 5-cc. vaccine bottles and stored in the ice box when not in use.

In order to determine whether the medium exudate was an influencing factor in the tests, a sufficient quantity of the fluid exuded from the sterile agar was collected and used for immunization, and the resulting antiserum was tested in series with the fungus antisera.

It was found unnecessary, as suggested by Link and Wilcox (7), to remove the lipoids from the fungus material in order to procure clear extracts. However the finely ground fungus material, on account of its cohesiveness, presented difficulty in the filtering process and necessitated the use of two or more Mandler filters.

Before administering the first injection to the rabbit to be immunized, 1.0 cc. of blood was taken from the marginal vein of one ear, and 0.5 cc. of the immunizing fluid was injected into the marginal vein of the other ear. The serum was removed from the blood clot and tested with the extract. None of the serum collected in this way gave a precipitin reaction. After the initial injection of 0.5 cc., seven subsequent intravenous injections of 1.0 cc. were given at intervals of one day. When the initial dose was increased to 1.0 cc. the animal sustained a severe shock which delayed further immunization for at least one week. Ten days after the last injection the animals were starved for 24 hr., chloroformed and bled from the heart by means of a sterile 50-cc. syringe with a No. 20 long needle. A method involving less experimental risk, especially when only one animal is available for immunization against an extract, would have been to take 10 to 20 cc. of blood from the ear vein and to repeat the operation after a day or so. No preservatives were added to the serum after its removal from the blood clot, or to the antigens. This necessitated most scrupulous aseptic technique.

In conducting the tests, serum dilutions were prepared with 0.85% sodium chloride solution in 0.5-cc. amounts, each of which was carefully overlaid with 0.5 cc. of antigen. Though this method of preparing the tests does not give the maximum titre, it does permit of more extensive tests from the same amount of antiserum than when the order of dilution is reversed. The controls set up with each test were: No. 1, undiluted immune serum plus saline, and No. 2, saline plus antigen. Positive controls occurred only with the antiserum produced from *U. avenae* extract. Readings were made after one hour's incubation at 37° C.; the racks were then removed to the ice box and a second reading was made the following morning. In a few instances the later readings gave a positive test in a higher dilution, but one positive reaction in a dilution of 1 : 80 became negative overnight. After reading the ring test satisfactory shake tests were not obtained.

Results

The agar-extract antiserum was found to be specific for its homologous antigen giving no cross reactions. It can be concluded then that the medium was not an influencing factor of variation in the reactions.

Table I shows the reactions of the immune sera prepared from the four monosporidial cultures supplied by Dr. Christensen. In each test the immune

TABLE I

ANTISERUM-EXTRACT REACTIONS

Fungal extracts (antigens)	Serum dilutions							Serum dilutions						
	Serum + antigen	1:5	1:10	1:20	1:40	1:80	1:160	Serum + antigen	1:5	1:10	1:20	1:40	1:80	1:160
	Sorosporium reilianum[C]							*Ustilago hordei*[C]						
S. reilianum[C]	+	+	+	+	+	+	0	—	—	—	—	—	—	—
U. hordei[C]	+	+	+	0	0	0	0	+	+	+	+	+	+	+
U. levis[C]	+	+	+	0	0	0	0	0	0	0	0	0	0	0
U. zeae[C]	+	+	+	0	0	0	0	0	0	0	+	±	0	0
U. hypodites	+	+	0	0	0	0	0	—	—	—	—	—	—	—
U. tritici	+	+	0	0	0	0	0	—	—	—	—	—	—	—
U. avenae	+	+	0	0	0	0	0	—	—	—	—	—	—	—
U. levis	+	+	0	0	0	0	0	—	—	—	—	—	—	—
U. zeae	+	+	0	0	0	0	0	—	—	—	—	—	—	—
Medium exudate	—	—	—	—	—	—	—	0	0	0	0	0	0	0
	Ustilago levis[C]							*Ustilago zeae*[C]						
S. reilianum[C]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. hordei[C]	+	+	+	+	±			0	±	0	0	0	0	0
U. levis[C]	+	+	+	+	+	+	±	0	0	0	0	0	0	0
U. zeae[C]	+	0	0	0	0	0	0	+	+	+	+	+	+	0
U. hypodites	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. tritici	±	±	0	0	0	0	0	+	+	+	+	+	±	0
U. avenae	±	±	0	0	0	0	0	+	+	+	+	+	±	0
U. levis	±	0	0	0	0	0	0	0	+	+	+	0	0	0
U. zeae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medium exudate	±	0	0	0	0	0	0	0	0	0	0	0	0	0

Note: *+ = definite precipitin ring; ± = doubtful; — = no test; 0 = negative.*

serum plus its homologous antigen gave a ring test in a higher dilution than with any of the three heterologous antigens; the difference in each instance showing a specificity which would permit species differentiation. Thus *U. zeae*[C] was completely specific. *U. levis*[C] immune serum gave two cross reactions, *i.e.*, *U. hordei*[C] (1:20) and *U. zeae*[C] (serum plus antigen). The serum of *U. hordei*[C] reacted with *U. zeae*[C] antigen (1:20). Cross reactions occurred in *S. reilianum* serum, with the three heterologous antigens (1 : 10).

As for the reactions of the remaining five antisera (Table II), the results indicate less specificity. *U. levis* antiserum showed positive with its homo-

TABLE II

ANTISERUM-EXTRACT REACTIONS

Antigens	Serum dilutions							Serum dilutions						
	Serum + antigen	1:5	1:10	1:20	1:40	1:80	1:160	Serum + antigen	1:5	1:10	1:20	1:40	1:80	1:160
	Ustilago hypodites							*Ustilago tritici*						
S. reilianum[C]	—	—	—	—	—	—	—	0	0	0	0	0	0	0
U. hordei[C]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. levis[C]	—	—	—	—	—	—	—	0	0	0	0	0	0	0
U. zeae[C]	—	—	—	—	—	—	—	+	+	+	0	0	0	0
U. hypodites	+	+	+	±	0	0	0	+	+	+	+	0	0	0
U. tritici	+	0	0	0	0	0	0	+	+	+	+	+	0	0
U. avenae	—	—	—	—	—	—	—	0	0	0	0	0	0	0
U. levis	—	—	—	—	—	—	—	0	0	0	0	0	0	0
U. zeae	—	—	—	—	—	—	—	+	+	+	0	0	0	0
Medium exudate	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Ustilago avenae							*Ustilago zeae*						
S. reilianum[C*]	+	+	+	±	±	0	0	0	0	0	0	0	0	0
U. hordei[C]	+	0	0	0	0	0	0	+	0	0	0	0	0	0§
U. levis[C]	±	0	0	0	0	0	0	0	0	0	0	0	0	0
U. zeae[C]	+	+	0	0	0	0	0	+	+	+	±	0	0	0§
U. hypodites†	+	+	+	+	+	±	0	+	+	+	+	0	0	0
U. tritici†	+	+	+	±	0	0	0	0	0	0	0	0	0	0
U. avenae	+	+	+	+	+	0	0	0	0	0	0	0	0	0
U. levis	+	+	+	+	0	0	0	+	+	+	0	0	0	0
U. zeae	+	+	0	0	0	0	0	+	+	+	+	0	0	0§
Medium exudate	0	0	0	0	0	0	0	—	—	—	—	—	—	•—
	Ustilago levis							Agar exudate						
S. reilianum[C]	0	0	0	0	0	0	0	—	—	—	—	—	—	—
U. hordei[C]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. levis[C]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U. zeae[C]	+	±	0	0	0	0	0	0	0	0	0	0	0	0
U. hypodites	+	+	0	0	0	0	0	—	—	—	—	—	—	—
U. tritici	+	+	+	0	0	0	0	—	—	—	—	—	—	—
U. avenae	0	0	0	0	0	0	0	—	—	—	—	—	—	—
U. levis	+	+	+	+	+	+	+	0	0	0	0	0	0	0
U. zeae	0	0	0	0	0	0	0	—	—	—	—	—	—	—
Medium exudate	—	—	—	—	—	—	—	+	+	+	+	+	+	+

* = ± *No. 1 control*; † = + *No. 1 control*; § = *negative on second reading.*

logous antigen when diluted 1 : 160 and gave cross reactions with the antigens of *U. tritici* and *U. hypodites* in dilutions of 1 : 10 and 1 : 5 respectively. *U. tritici* antiserum showed positive in a dilution of 1 : 80 with its own antigen and reacted also with *U. hypodites* (1 : 20), *U. zeae*^{C} (1 : 10), and *U. zeae* (1 : 10). The reactions in this instance as in the case of *U. levis* antiserum may be considered definite enough in differentiating the species. The immune serum of *U. avenae* gave a homologous reaction of 1 : 40 and a cross reaction with *U. levis* (1 : 20). The only doubtful controls were encountered in this test. It was felt that the results of the *U. hypodites* tests were inadequate because of having insufficient antiserum to complete the tests. The necessity of immunizing animals in duplicate is well brought out at this point. The low titre of *U. zeae* antiserum (1 : 20) may be explained by the failure of the animal to respond to immunization (4), or to the use of a less potent extract in producing the antiserum, but all of these tests need to be duplicated before deductions can be drawn. However, this seems undesirable inasmuch as the writer's results are in general agreement with those of Link and Wilcox, from which it seems evident that the precipitin ring test is not sufficiently specific for the purpose in mind, namely, the differentiation of closely related species and physiological forms. There is a suggestion in one result that this method might prove valuable in indicating the closer affinities among groups of doubtful species or genera.

Acknowledgments

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A DETERMINATION OF THE DISTORTION IN A NUMBER OF AIR CAMERA LENSES[1]

BY R. H. FIELD[2]

Abstract

For map plotting purposes it is essential to know the principal distance (focal length) of air cameras, preferably for rays making various angles with the lens axis. A compact apparatus has been developed for making the necessary measurements and has been used for investigating the lens distortion in a number of air cameras. This method of finding distortion has the advantage that it takes into account the effect of the glass plate fitted in film cameras to maintain the film flat during exposure.

The distortion is given as the linear displacement in the plane of the photograph. It is felt that in this form the results are of more interest to those using air photographs. By simple calculations the figures can be changed to give the distortion in the forms more usual in the treatment of lens design.

Air photography makes severe demands on photographic lenses. The speed at which aircraft move relatively to the ground, the need for a maximum angular field of view in the interest of economy and the necessity for employing filters have resulted in air cameras being fitted with lenses having F/D ratios of four or less.

In applying the process of mapping from air photographs the most important fault in these lenses is distortion (2, pp. 48 and 63). Distortion results in a departure from the ideal perspective condition that point images in the picture plane subtend the same angle at the perspective centre as the corresponding points on the ground.

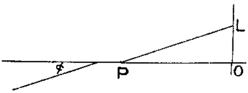

FIG. 1. *Diagram illustrating the path of a light ray in an air camera.*

P, Fig. 1, is the rear node of a lens and OL the plane of the negative, O being the foot of the perpendicular from P (*i.e.*, the "principal point" of the camera or photograph). A bundle of rays from a distant point makes an angle ϕ with OP and gives rise to an image L on the negative. In the ideal case angle OPL is equal to ϕ. Practically, if the distance OP be computed from the relation

$$OP = OL \cot \phi, \tag{1}$$

OL and ϕ being measured, the value of OP is found to vary with ϕ. As the position of the picture plane is fixed, it can be assumed that the position of the rear node, P, is not constant. For optical calculations the focal length of a lens is usually taken as the value of OP computed from Equation (1) when OL is infinitely small, while for map plotting purposes it is general to determine an average value of OP for the whole or a considerable portion of the angular field covered by the photograph. In this case the residuals between the value chosen and the actual value of OP at specific points are usually smaller (2, p. 64).

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Contribution from the Physical Testing Laboratories, Division of Physics, National Research Laboratories, Ottawa, Canada.

[2] *Supervisor, Physical Testing Laboratories, National Research Council, Ottawa.*

The practical effect of the variation in the value of the principal distance, *OP*, is to give rise to changes in the scale of the photograph which depend on the zone in which images fall. If some particular value, *OP*, is taken for the principal distance, and is assumed to apply to the whole photograph, images where the true principal distance is $OP + \delta$ will be displaced radially on the photograph by an amount $\delta \tan \phi$, to a first approximation, from their nominal positions.

Each year, after overhaul, the principal distances of the cameras used by the Royal Canadian Air Force are determined in this laboratory. For such checks the observations need be made for one or two zones only, but on one occasion for most of the cameras they have been extended to cover the whole range of the picture. From these measurements some interesting data have been acquired regarding the distortion in lenses of differing design and focal length.

In the method which has been developed in this laboratory for determining the principal distance, a rectangular glass plate engraved with two diametral lines, crossed by short lines accurately placed at centimetre intervals, is held against the glass focal plane plate of the camera with the aid of simple devices, which vary slightly with different forms of camera. The angle ϕ (Fig. 1) is then measured with the aid of a theodolite reading to single seconds, measurements being made (in the special determinations) at each centimetre from *O* to the edge of the camera opening.

Fig. 2 is a sketch of the apparatus. The camera is mounted with the axis of the lens horizontal on a stand supported by three footscrews; two of these rest in grooved rails and the third is supported on a plane rail. Two round rails, approximately perpendicular to those supporting the camera-stand,

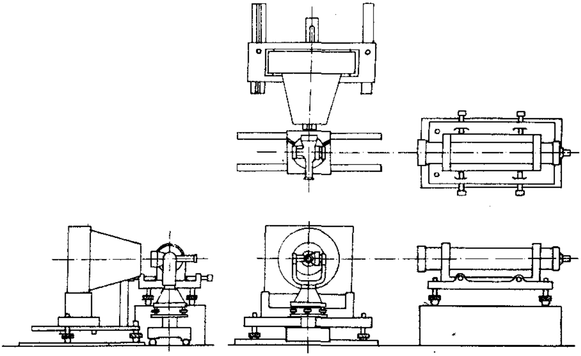

FIG. 2. *Plan and elevations of apparatus for determining the lens constants of air cameras.*

bear a cast iron sliding member which supports the theodolite. Both the camera and theodolite stands are sufficiently massive that, with ordinary care on the part of the observer, observations are concordant and there is no necessity for clamping. Thus the theodolite can be placed quickly in the correct position to sight through the camera lens on to one of the graduation marks of the glass plate.

A collimator, serving as a reference azimuth for the angles measured, is arranged almost collinear with the direction of the rails supporting the theodolite, at about the same height as the axes of the theodolite telescope and camera, when all three axes are horizontal. The various adjustments required can be made rapidly to the necessary degree of accuracy. Concrete piers support the whole apparatus in a room subject to very small temperature fluctuations.

To simplify calculations the glass plate is first located with the camera focal plane plate accurately leveled over a mercury surface. Using an autocollimating eyepiece the plate is adjusted until the intersection of the diametral lines, seen through the eyepiece, coincides with the image reflected from the mercury. The intersection should then be at the principal point. It is stated (1, p. 54) that this method is liable to error arising from centering errors in the lens components. An error of this nature should show itself by differences in the angles subtended by the nearer marks equally spaced on either side of the intersecting diametral lines (1, p. 26). In the lenses so far examined no appreciable effect of this nature has been found.

After the plate has been located and clamped, the camera is placed on its support and the lens axis and horizontal line on the plate are brought to true horizontality with the aid of the footscrews, the operation being controlled by observations through the theodolite telescope. Horizontal angles between the graduation marks and the collimator axis are then measured, one mark at a time. For each measurement the telescope is adjusted so as to point close to the centre of the lens opening. As the marks approach the edges this adjustment is best controlled by viewing the exit pupil of the telescope through a small magnifier and moving the theodolite until the meniscus formed by the lens aperture appears centrally placed.

Check observations are made on the intersection of the diametral lines at the end of each set, and, more nearly to equalize the weights, additional pointings are made on the graduations nearer the intersection, where the measured angles are small and the computed distances subject to greater error.

In each set of observations angles are measured on both diameters of the graduated plate, *i.e.*, in four directions outward from the principal point, and the mean value of the principal distance is computed for each zone from Equation (1). For one or two lenses a small systematic difference was found between some of the measurements along different radii, and it was concluded that the distortion was not quite symmetrical.

The results of the observations so far concluded are shown in Table I. In each case the mean value of the principal distance was the mean of the values

computed for the eight groups of points, 1 to 8 cm. distant from the principal point. Distortion was computed from the difference between this mean and the actual computed principal distance for the radius in question. A plus sign signifies distortion away from the centre.

TABLE I

DISTORTION IN MICRONS IN THE PLANE OF THE NEGATIVE

No.	Principal distance, mm.	Distance from principal point, mm.											F/D
		10	20	30	40	50	60	70	80	90	100	110	
"Xpress" lenses													
119798	208.93	+ 5	+ 8	+10	+ 4	− 7	−14	−30	−42	−52	−58	−84	4.5
123427	209.56	+ 1	+ 5	+ 9	− 2	− 2	−14	− 7	−23	−52	−53	−58	4.5
123428	209.74	+ 3	+ 8	+ 7	+ 4	− 7	−14	−17	−23	−26	−62	−58	4.5
123429	209.73	+ 3	+ 4	+ 4	− 2	− 2	−12	−10	−23	−48	−62	−63	4.5
123430	209.63	+ 6	+ 8	+10	+ 2	−14	−26	−27	−31	−65	−67	−79	4.5
126715	211.06	+ 4	+ 4	+ 6	− 4	− 9	− 8	−10	−19	−42	−42	−41	4.5
126718	210.99	+ 5	+ 7	+ 7	0	− 5	−20	−20	−26	−47	−75	−145	4.5
126719	211.23	+ 4	+ 3	+ 8	+ 2	− 7	−14	−16	−23	−38	−66	−78	4.5
126727	211.54	+ 3	+ 5	+ 6	0	−12	−11	−13	−23	−34	−61	−67	4.5
123447	303.37	− 1	+ 1	+ 6	+ 3	0	0	− 7	−13	−24	−40	−54	4
126889	511.70	+ 1	+ 5	+ 5	+ 3	0	− 2	−14	−23	−37	−55	−64	4
"Dagor" lenses													
396769	210.01	− 4	− 4	− 1	0	+ 2	0	+13	+23	+43	+62	+79	6.8
398305	210.50	− 5	− 5	− 9	− 6	+ 2	+ 9	+20	+38	+60	+86	+115	6.8
399219	209.29	−.2	− 5	− 7	−10	− 2	+ 3	+20	+38	+52	+77	+111	6.8
399221	207.25	− 4	−10	− 4	− 4	+ 7	+ 9	+24	+38	+60	+72	+132	6.8
399223	209.41	− 2	− 7	− 9	− 4	+ 5	+12	+17	+39	+48	+86	+111	6.8
399224	208.40	+ 1	− 2	− 3	−13	−17	+12	+20	+31	+65	+38	+16	6.8
399241	206.33	− 3	− 3	− 4	+ 2	− 5	0	+13	+27	+52	+82	+106	6.8
399249	208.56	− 4	− 5	− 7	− 2	+ 7	+ 9	+13	+23	+43	+62	+84	6.8
399253	207.45	− 3	− 3	− 9	− 4	+ 7	+ 6	+10	+23	+17	+48	+84	6.8
751117	208.62	− 2	− 3	− 4	− 2	− 2	0	+ 7	+31	+65	+86	+127	6.8
751131	209.02	0	− 2	− 9	−19	− 5	+14	+13	+31	+48	+67	+100	6.8
752160	209.21	− 4	− 7	− 4	− 2	+ 5	+ 9	+17	+27	+30	+43	+79	6.8
"Tessar" lenses													
726727	249.66	− 1	+ 5	+ 6	+10	+ 2	+ 2	−11	−38	−76	−132	−172	4.5
736720	249.64	+ 2	+ 6	+ 8	+ 5	+ 2	− 5	− 8	−48	−101	−160	−246	4.5
736721	249.79	+ 1	+ 5	+ 6	+ 6	+ 6	− 5	−20	−42	−90	−124	−198	4.5
737748	250.26	0	+ 4	+ 6	+ 5	+ 2	− 2	−11	−35	−79	−136	−220	4.5
422415	298.52	+ 3	+ 7	+ 9	+ 9	+ 2	− 8	−26	−48	−69	−117	−181	4.5
479890	297.91	− 1	0	+ 6	+12	+ 3	0	−12	−24	−58	−92	−146	4.5
484152	296.94	+ 4	+ 7	+11	+ 3	+ 7	−12	−31	−60	−104	−160	−224	4.5
512148	298.94	+ 3	+ 6	+10	+ 4	+ 2	−10	−26	−54	−87	−137	−199	4.5
642351	298.71	+ 2	+ 4	+ 6	+ 7	0	− 6	−16	−32	−54	−87	−147	4.5
660756	298.48	+ 1	0	+ 3	0	+ 3	− 2	− 5	−19	−30	−60	−87	4.5
664269	298.28	+ 2	+ 2	+ 6	+ 1	− 2	− 4	−16	−29	−33	−50	−114	4.5
674010	299.50	+ 2	− 1	+ 3	0	+ 2	− 4	− 5	−13	−36	−33	−40	4.5
674012	299.38	− 1	+ 3	+ 4	0	+ 2	0	0	− 5	−12	−26	−33	4.5
674013	298.71	− 1	+ 1	+ 3	+ 4	+ 2	+ 2	− 5	−11	−18	−44	−63	4.5
674015	298.95	+ 3	+ 4	+ 3	− 3	0	− 4	− 5	−29	−30	−33	−62	4.5
814523	297.63	+ 1	+ 5	+ 1	− 1	+ 3	0	−10	−16	−55	−85	−131	4.5
814570	296.97	+ 1	+ 3	+ 4	+ 4	0	0	−12	−27	−46	−82	−120	4.5
814571	297.58	+ 3	+ 4	+ 6	+ 4	0	−10	−17	−30	−43	−71	−105	4.5
670183	500.58	0	− 1	− 3	− 2	+ 4	− 1	0	+ 5	+ 2	−4	+ 4	5

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THE SOUND FIELD OF MEMBRANES AND DIAPHRAGMS

II. THE POWER EMITTED BY CIRCULAR MEMBRANES[1]

By R. Ruedy[2]

Abstract

Accurate formulas are presented showing the power radiated by a stretched circular membrane vibrating with small amplitudes and an arbitrary number of nodal circles or diameters. When the nodes are circular, the sound emitted is expressed as a sum of the products of simple functions of ka, $J_0(2ka)$, $J_1(2ka)$ by the definite integral of $y^{2r}/(\kappa^2a^2/k^2a^2-1+y^2)^2$ taken between the limits zero and unity. For values of $ka=2\pi a/\lambda$ between zero and two, where the radius a is taken as 10 cm., the membrane vibrating in its fundamental mode emits between three and four times as much power as the piston having the same maximum velocity at its centre. The first and second overtones have about the same strength as the corresponding fundamental frequencies, so the power radiated increases less rapidly with frequency than when nodes are absent.

Introduction

The strength of the sound emitted by diaphragms and membranes was studied when telephone receivers were developed. In view of the fact, however, that modern large-sized reproducers of speech and music, and the lines used for transmitting them over long distances, are expected to convey to the hearer a much wider range of frequencies than is necessary in reproducing simple conversation from person to person, it is of practical interest to study the question more thoroughly than before (1–4).

When the elements of a circular surface which is set into an infinite wall or baffle perform harmonic vibrations normal to the surface according to a function $w(r, \phi)$, the total power emitted from one side can be computed by two different methods. The usual solution is to put the rate at which the source does work equal to the sum of the products of pressure variation by the particle velocity at the surface, using root mean square values for both, the pressure variation at any one point being itself the resultant of the pressure exerted upon the element by all the other points (Lord Rayleigh, I. B. Crandall). Except for perfectly plane waves the total pressure is found to possess a component in phase with the velocity, proportional to the power radiated, and a lagging component, proportional to the mass of the air set into motion (so-called accession to inertia).

On the other hand, when air damping is neglected, the power of the source in producing sound waves must be the same when integrated over any distant spherical surface of radius R, having the source of radius a at its centre, and equal to the mean sum of the products of alternating pressure $p=-s\partial\Phi/\partial t$ and radial velocity $v=\partial\Phi/\partial r=kp/s$ over the entire surface of one side of the disk, Φ being the velocity potential. Here s is the density in

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grams per cubic centimetre and $k = 2\pi/\lambda$. With

$$\Phi = -\frac{\epsilon^{i(pt-kR_o)}}{2\pi R_o}\int_{\phi=0}^{2\pi} d\phi \int_{\gamma=0}^{\pi/2} ip\dot{w}(r,\phi)\,\epsilon^{ikl} r\,dr \sin\gamma\,d\gamma,$$

γ being the angle which the chosen direction forms with the normal to the disk, the power emitted by the source becomes

$$E = \frac{c\rho}{2\lambda^2}\int_0^{2\pi} d\phi \int_0^{\pi/2} d\gamma \sin\gamma \left[\int_0^{a}\int_{\phi=0}^{2\pi} \dot{w}(r,\phi)\,r\,dr\,d\phi\,\epsilon^{ikl}\right]^2,$$

and the mass of the air to be displaced is $-c\rho/4\pi i\lambda R_o$ multiplied by the fourfold integral. The length l is equal to $r \sin\gamma \cos\phi$.

The Rigid Piston

If we take the rigid piston as a basis of comparison, all the surface elements possess the same amplitude, $\dot{W}_o$, of velocity and the same phase, and since

$$J_o(u) = \frac{2}{\pi}\int_0^{\pi/2} \cos(u\cos\phi)\,d\phi = \frac{1}{2\pi}\int_0^{2\pi}\cos(u\cos\phi)\,d\phi$$

and

$$\int_0^{r} r^{n+1}J_n(r)\,dr = r^{n+1}J_{n+1}(r),$$

it follows that as far as real quantities are concerned

$$\int_0^{r=a}\int_{\phi=0}^{2\pi}\epsilon^{ikl} r\,dr\,d\phi = \int_0^{a}\int_0^{2\pi} r\,dr\cos(kr\sin\gamma\cos\phi)\,d\phi = 2\pi\int_0^{r=a} rJ_o(kr\sin\gamma)\,dr = 2\pi a^2\,\frac{J_1(ka\sin\gamma)}{ka\sin\gamma}.$$

Hence

$$E = \frac{4\pi^2 a^4}{\lambda^2}c\rho\frac{\dot{W}_o^2}{2}\int_0^{2\pi} d\phi\int_0^{\pi/2} d\gamma\sin\gamma\,\frac{J_1^2(ka\sin\gamma)}{(ka\sin\gamma)^2}.$$

Taking into account the relation

$$J_n^2(y) = \sum_{m=0}^{\infty}(-1)^m\frac{\Gamma(2n+2m+1)}{\Gamma(m+1)\Gamma(2n+m+1)\Gamma^2(n+m+1)}\left(\frac{y}{2}\right)^{2n+2m},$$

$$J_1^2(ka\sin\gamma) = \sum_{m=0}^{\infty}(-1)^m\frac{\Gamma(2m+3)(ka)^{2m+2}}{\Gamma(m+1)\Gamma(m+3)\Gamma^2(m+2)}\,\frac{\sin^{2m+2}\gamma}{2^{2m+2}},$$

where the symbol Γ indicates the gamma function, which reduces in the present case to mere factorials, therefore

$$\int_0^{\pi/2}\frac{J_1^2(ka\sin\gamma)}{k^2a^2\sin^2\gamma}\sin\gamma\,d\gamma = \sum_{m=0}^{\infty}(-1)^m\frac{\Gamma(2m+3)(ka)^{2m}}{2^{2m+2}\Gamma(m+1)\Gamma(m+3)\Gamma^2(m+2)}\int_0^{\pi/2}\sin^{2m+1}\gamma\,d\gamma.$$

As $(2m+1)$ is an odd number the integral becomes equal to $2^m\Gamma(m+1)/1.3.5\ldots(2m+1)$, and finally

$$E = \pi a^2 c\rho k^2 a^2 \frac{\dot{W}_o^2}{2}\left(\frac{1}{1.2} - \frac{k^2a^2}{1.2^2 3} + \frac{k^4a^4}{1.2^2 3^2 4} - \frac{k^6a^6}{1.2^2 3^2 4^2 5}\cdots\right)$$

or

$$E = c\rho\pi a^2\left(1 - \frac{J_1(2ka)}{ka}\right)\frac{\dot{W}_o^2}{2},$$

a formula which is in agreement with the result obtained by the first method.

Power Emitted by the Circular Membrane

In the case of the circular membrane, or drumhead, firmly clamped at the edge, the directional properties of the pressure amplitude are determined by the derivative of the velocity potential Φ with respect to time (4). For even values of n

$$\Phi = -2\pi a^2\kappa_{nm}aJ_{n-1}(\kappa_{nm}a)\frac{A'_{nm}}{2\pi R_o}\cos n\phi\,\frac{J_n(ka\sin\gamma)}{\kappa_{nm}^2a^2 - k^2a^2\sin^2\gamma},$$

leaving out the factor $e^{i(pt-kR_o)}$; and the main difference with regard to the piston is the fraction containing J_n which must be squared and integrated over the entire hemisphere of radius R_o. This gives

$$E = 4\pi^2a^4\kappa_{nm}^2a^2\frac{c\rho}{2\lambda^2}J_{n-1}^2(\kappa_{nm}a)A'^2_{nm}\int_{\phi=0}^{2\pi}d\phi\cos^2 n\phi\int_{\gamma=0}^{\pi/2}d\gamma\sin\gamma\,\frac{J_n^2(ka\sin\gamma)}{(\kappa_{nm}^2a^2 - k^2a^2\sin^2\gamma)^2}.$$

With $x = \sin\gamma$ and $dx = \sqrt{1-x^2}\,d\gamma$, and taking into account the relation

$$\int_0^{2\pi}\cos^2 n\phi\,d\phi = \int_0^{2\pi}\sin^2 n\phi\,d\phi = \pi\quad,$$

except when $n = 0$, in which case the first integral is equal to 2π,

$$E = \frac{\kappa_{nm}^2a^2}{k^2a^2}c\rho\pi a^2 J_{n-1}^2(\kappa_{nm}a)\frac{A'^2_{nm}}{2}\int_0^1\frac{xJ_n^2(kax)dx}{\left(\frac{\kappa^2a^2}{k^2a^2} - x^2\right)\sqrt{1-x^2}}.$$

Now

$$\int\frac{xJ_n^2(kax)dx}{\left(\frac{\kappa^2a^2}{k^2a^2} - x^2\right)^2\sqrt{1-x^2}}$$

$$= \sum_{m=0}^{\infty}(-1)^m\frac{\Gamma(2n+2m+1)}{\Gamma(m+1)\Gamma(2n+m+1)\Gamma^2(n+m+1)}\left(\frac{ka}{2}\right)^{2(n+m)}\int\frac{x^{2n+2m}x\,dx}{\left(\frac{\kappa^2a^2}{k^2a^2} - x^2\right)\sqrt{1-x^2}}.$$

With $y^2 = 1 - x^2$ the last integral becomes

$$\int_0^1 \frac{(1-y^2)^{n+m}}{\left(\left(\frac{\kappa^2 a^2}{k^2 a^2}-1\right)+y^2\right)^2} dy$$

$$= \int_0^1 \frac{1 - \frac{(n+m)}{1!} y^2 + \frac{(n+m)(n+m-1)}{2!} y^4 - \frac{(n+m)(n+m-1)(n+m-2)}{3!} y^6 \dots}{(q^2+y^2)^2} dy.$$

Depending upon whether $\frac{\kappa^2 a^2}{k^2 a^2} \lessgtr 1$, when s is an even number.

$$\int \frac{y^s}{(q^2+y^2)^2} = \frac{(s-1)}{q^2+y^2}\left(\frac{y^{s-1}}{(1-s)(3-s)} - \frac{q^2 y^{s-3}}{(3-s)(5-s)} + \frac{q^4 y^{s-5}}{(5-s)(7-s)} - \frac{q^6 y^{s-7}}{(7-s)(9-s)} \dots \pm \frac{q^{s-2} y}{2}\right) \pm \frac{(s-1) q^s}{2q^2\sqrt{\pm q^2}} \begin{matrix}\tan^{-1} \\ \tanh^{-1}\end{matrix} \frac{y}{\sqrt{\pm q^2}}$$

where, for values of s greater than two, and $s/2$ an odd number, it is correct to use the plus sign before the last term and the opposite sign for the last term but one, while the reverse is true when $s/2$ is even. In the last term the positive sign applies to $\tan^{-1}$ and $\kappa^2 a^2/k^2 a^2 \lessgtr 1$; the negative sign goes with $\tan h^{-1}$ and $\kappa^2 a^2/k^2 a^2 \lessgtr 1$. If desired $\tan h^{-1}(1/q)$ may be replaced by $1/2 \log (x-q)/(x+q)$. Finally, when $\kappa^2 a^2/k^2 a^2 = 1$, only the first term of the equation is required. Introducing the limits,

$$\int_0^1 \frac{y^o}{(q^2+y^2)^2} dy = \frac{1}{2q^2}\left(1 + \frac{q^2+1}{q} \tan^{-1} \frac{1}{q}\right) \frac{k^2 a^2}{\kappa^2 a^2},$$

$$-\int_0^1 \frac{y^2}{(q^2+y^2)^2} dy = \left(\frac{1}{2} - \frac{q^2+1}{q} \tan^{-1} \frac{1}{q}\right) \frac{k^2 a^2}{\kappa^2 a^2},$$

$$\int_0^1 \frac{y^s}{(q^2+y^2)^2} dy = (s-1) \frac{k^2 a^2}{\kappa^2 a^2}\left\{\frac{1}{(1-s)(3-s)} - \frac{q^2}{(3-s)(5-s)} + \frac{q^4}{(5-s)(7-s)} + \dots \pm \frac{q^{s-2}}{2q^2} \pm \frac{q^s (q^2+1)}{\pm 2q^2\sqrt{\pm q^2}} \begin{matrix}\tan^{-1} \\ \tan h^{-1}\end{matrix} \frac{1}{\sqrt{\pm q^2}}\right\}$$

Where only circular nodes exist ($n=0$), the number s equals m, and

$$E_o = \pi a^2 c\rho J_1^2(\kappa_{om} a) \dot{A}_{om}^2 \sum_{m=o}^{\infty} (-1)^m \frac{\Gamma(2m+1)}{\Gamma^4(m+1)} \frac{ka^{2m}}{2^{2m}} \frac{\kappa_{om}^2 a^2}{k_{om}^2 a^2} \int_0^1 \frac{1 - \frac{my}{1!} + \frac{m(m-1)y^4}{2!} \dots \pm y^m}{(q^2+y^6)^2} dy,$$

and where $n=2$ (two nodal diameters), $s=m+2$, so that

$$E_2 = \pi a^2 c\rho J_1^2(\kappa_{2m} a) \frac{\dot{A}_{2m}^2}{2} \sum_{m=o}^{\infty} (-1)^m \frac{\Gamma(2m+5)(ka/2)^{2m+4}}{\Gamma(m+1)\Gamma(m+5)\Gamma^2(m+3)} \frac{\kappa_{2m}^2 a^2}{k_{2m}^2 a^2}$$

$$\times \int_0^1 \frac{1 - \frac{(m+2)y^2}{1!} + \frac{m(m+2)(m+1)y^4}{2!} + \dots \pm y^{m+2}}{(q^2+y^2)^2} dy$$

and so on for a higher number of diametral nodes. The power contained in overtones in any mode of vibration is obtained by inserting the proper value of κ_{nm}, the integral remaining the same for any overtone for a given number of diametral nodes.

Discussion of Results

Since the resonance frequencies f_{nm} of the membrane are given by $f_{nm} = \frac{\kappa_{nm}a}{2\pi a}\sqrt{\frac{T}{s'}}$, where Tdl is the tension in dynes across a straight line of length dl drawn anywhere upon the membrane, s' the mass in grams per sq. cm. and $(\kappa_{nm}a)$ the solution of the equation $J_n(\kappa a) = 0$ (see Table I), whatever the frequency, the following relation holds

$$cka/\kappa a = \sqrt{T/s'}$$

or

$$ka/\kappa a = af_{oo}/12754,$$

in other words equals a constant value for a given tension of the membrane.

In particular, ka equals unity for $\sqrt{T/s'} = c/\kappa a$, and $ka = \kappa a$ for $\sqrt{T/s'} = 3.33 \times 10^4$. As f_{oo} is equal to $0.383\sqrt{T/s'}/a$, it follows that a membrane of 10 cm. radius, for which $k_{oo}a$ is made equal to unity (or $ka/\kappa a = 0.416$ and $q^2 = 4.784$), possesses a resonance frequency f_{oo} of 530 cycles per second corresponding to a wave-length in air of about 63 cm., and a membrane of the same dimensions, but stretched so as to give a value $k_{oo}a$ of 2.405, that is, equal to $\kappa_{oo}a$, and q^2 equal to zero, has a resonance frequency of about 1280 cycles per second ($\lambda = 26$ cm.). But these values involve the highest tensions safely applicable to membranes of known materials, and in general, therefore, the value of $\kappa_{oo}a/k_{oo}a$ is larger than unity, q^2 remaining positive as the resonance frequency f_{oo} is varied by changing the tension applied to the membrane. A case in point is the modern type of electrostatic membrane speaker in which an aluminium alloy of exceptional strength is used. With a radius of 19 cm., a thickness of 0.0016 cm. and a total weight of five grams, the fundamental frequency observed is 130 cycles per second.

TABLE I

SOLUTIONS $\kappa_{nm}a$ OF THE MEMBRANE EQUATION $J_n(\kappa a) = 0$

—	$m = 0$	$m = 1$	$m = 2$	$m = 3$	m
$n = 0$	2.405	5.520	8.654	11.792	$(m+\frac{3}{4})\pi$
$n = 1$	3.832	7.016	10.137	13.233	$(m+\frac{5}{4})\pi$
$n = 2$	5.135	8.417	11.620	14.796	$(m+\frac{7}{4})\pi$

Note :— m, number of circular nodes; n, number of nodal diameters.

(a) Membrane Vibrating with its Fundamental Frequency

In the case of the membranes for which, as indicated above, the value of $k_{m0}a$ is smaller than unity, the expression (κ^2a^2/k^2a^2-1) will be positive for all values of κ_{om}, and the power radiated in ergs at various frequencies is, apart from the factor $\pi a^2 c\rho J_1^2(\kappa a)\dot{A}^2_{oo}$, given by

$$\begin{aligned}\Sigma = &\left[1-\frac{2!}{1!^4}\left(\frac{\pi a}{\lambda}\right)^2+\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-1.\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+1.\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\right]\left[\frac{1}{2q^2}+\frac{q^2+1}{2q^3}\tan^{-1}\frac{1}{q}\right]\\
&+\left[-\frac{2!}{1!^4}\left(\frac{\pi a}{\lambda}\right)^2+2.\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-3.\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+4.\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots\right]\left[\frac{1}{2}-\frac{q^2+1}{2q}\tan^{-1}\frac{1}{q}\right]\\
&+\left[\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-3.\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+6.\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots\right]\left[1+\frac{3}{2}q^2-\frac{3}{2}\frac{q^2+1}{q}\tan^{-1}\frac{1}{q}\right]\\
&+\left[-1.\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+8.\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots\right]\left[-\frac{1}{3}+\frac{5}{3}q^2+\frac{5}{2}q^4-\frac{5}{3}q^3(q^2+1)\tan^{-1}\frac{1}{q}\right]\\
&+\left[1.\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots\right]\left[\frac{1}{5}-\frac{7}{5.3}q^2+\frac{7}{3.1}q^4+\frac{7}{2}q^6-\frac{7}{2}q^7(q^2+1)\tan^{-1}\frac{1}{q}\right]\end{aligned}$$

for all values of $\pi a/\lambda$ smaller than 1.202 and modes of vibration involving circular nodes only. In evaluating the second bracket in each line advantage may be taken of the recurrence formula

$$\int\frac{y^s}{(q^2+y^2)^2}=-\frac{1}{(3-s)(q^2+1)}+\frac{s-1}{3-s}q^2\int\frac{y^{s-2}}{(q^2+y^2)^2}\,dy\,.$$

The infinite series occurring in the first bracket may be replaced if desired by the following simple functions of πa, $J_0(ka)$ and $J_1(ka)$:

$$1-\frac{2!}{1!^4}\left(\frac{\pi a}{\lambda}\right)^2+\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots=J^2_o(ka)$$

$$\begin{aligned}\frac{2!}{1!^4}\left(\frac{\pi a}{\lambda}\right)^2+2\,\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-3\,\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+4\,\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots&=\frac{\pi a}{\lambda}\frac{dJ_o^2(ka)}{d(ka)}\\
&=-kaJ_o(ka)J_1(ka)\end{aligned}$$

$$\begin{aligned}\frac{4!}{2!^4}\left(\frac{\pi a}{\lambda}\right)^4-3\,\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+6\,\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots&=\frac{1}{2}\left(\frac{\pi a}{\lambda}\right)^3\frac{d}{d(ka)}\frac{\lambda}{\pi a}\frac{dJ_o^2(y)}{dka}\\
&=\frac{ka}{4}\left\{ka\left(J_1^2(ka)-J_o^2(ka)\right)+2ka\,J_1J_o\right\}\end{aligned}$$

$$-\frac{6!}{3!^4}\left(\frac{\pi a}{\lambda}\right)^6+8\,\frac{8!}{4!^4}\left(\frac{\pi a}{\lambda}\right)^8\cdots=\frac{ka}{1.2}\left(J_1J_o(2k^2a^2-4)-3kaJ_1^2+2kaJ_o^2\right)$$

and the next two series are

$$\begin{aligned}\frac{1}{4}\left(\frac{\pi a}{\lambda}\right)^7\frac{d}{d(ka)}&\left(\frac{1}{3\pi a/\lambda}\frac{d}{d(ka)}\frac{1}{2\pi a/\lambda}\frac{d}{dka}\frac{1}{\pi a/\lambda}\frac{dJ_o^2(ka)}{d(ka)}\right)\\
&=\frac{ka}{48}\left[(12-9k^2a^2)J_1J_o+(k^3a^3-6ka)J_o^2+(11ka-k^3a^3)J_1^2\right]\end{aligned}$$

and

$$\frac{10!}{5!^4}\left(\frac{\pi a}{\lambda}\right)^{10}+\cdots=\frac{ka}{480}\left[(-96+88k^2a^2-4k^4a^4)J_1J_o+(48ka-13k^3a^3)J_o^2+(-100\,ka+15\,k^3a^3)J_1^2\right]$$

However, owing to the limited accuracy of the values of the Bessel functions found in the tables, when the variable *ka* is arbitrary there is little advantage in using the finite expressions in place of the series development except in the case of the first three or four expressions and small values of *ka*.

For very long waves, the power emitted on one side of the membrane becomes

$$E_{\infty} = \pi a^2 c\rho \frac{J_1^2(\kappa a)}{\kappa^2 a^2} \dot{A}_{oo}^2 = 1.864\, \pi a^2 c\rho \dot{A}_{oo}^2 \text{ ergs per sec.}$$

or over three times as much as would be obtained with a rigid piston having the same maximum velocity as the membrane possesses at its centre ($\dot{A} = \dot{W}$). When the tension applied to the membrane is increased and with it the resonance frequency, the power radiated assumes increasingly large values, as shown by Table II, which also shows for comparison the power radiated by the rigid piston of 10 cm. radius. (To find the power in absolute units (ergs), it is necessary to multiply the values given by 314.159 $c\rho\dot{A}^2$, where $c = 3.4 \times 10^4$ cm., $\rho = 0.0012$ gm. per cc. at 18° C. and 760 mm. Hg, and $\dot{A}$ is the maximum velocity or 6.28 f times the maximum amplitude, usually a few mm.) For all values of *ka* larger than 0.1 and smaller than 2, the piston emits three or four times less power than the membrane.

TABLE II

POWER RADIATED BY A MEMBRANE OF 10 CM. RADIUS AT VARIOUS RESONANCE FREQUENCIES.

—	$ka = 0.05$	$ka = 0.10$	$ka = 0.20$	$ka = 0.40$	$ka = 0.66$	$ka = 1$	$ka = 2$
$m = 0$	0.0025	0.0081	0.034	0.153	0.411	0.896	2.51
$m = 1$	—	—	—	—	0.075	0.17	—
$m = 2$	—	—	—	—	0.03	0.07	—
Piston	—	0.0025	0.01	0.039	0.105	0.21	0.562

NOTE: m = *number of circular nodes.*

(*b*) *Membranes Vibrating with Several Circular Nodes*

The formula for the sound emitted by the membrane vibrating with its fundamental frequency also applies to the membrane vibrating with one or two circular nodes, except that the values of *ka* must be increased, in accordance with the higher frequency of the overtones, by the factor 2.29 for the first, and by the factor 3.6 for the second overtone. The result thus obtained shows that the power with which the harmonics are emitted between $ka = 0$ and $ka = 2$ is practically the same as has been found for the corresponding fundamental, so it increases less rapidly with frequency than it does in the fundamental mode of vibration. This decrease is to be expected in view of the opposition in phase shown by points situated at either side of the circular node.

A more complete discussion will be presented later together with the results obtained with diaphragms (thin plates).

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REVIEWS AND NOTES

A Thermostat for Temperatures between 5° and 20° C.

Constant temperatures lower than room temperature are frequently required, but few convenient means of producing them are available. The apparatus described here (Fig. 1) represents an adaptation of a thermostat described previously (1), and has been successfully employed in this laboratory for maintaining small objects at any constant temperature in this range. The essential part is a small Dewar flask (12 cm. in length) with a relatively wide clearance between the walls. This space is filled with mercury, which acts as the thermostat liquid and at the same time constitutes the thermoregulator, maintaining the space *A* at a constant temperature. A heating coil of 5 to 10 ohms resistance is wound directly on the glass (Pyrex). No relays or special circuits are required as the contact point simply short circuits the heating coil through the 50 watt lamp. No perceptible spark appears at the contact. The space *B* is filled with asbestos and is considerably larger in proportion to the Dewar than is shown in the diagram.

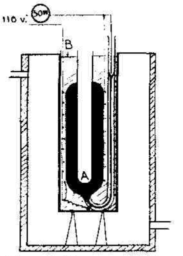

FIG. 1. *Thermostat for temperatures between 5° and 20° C.*

The whole apparatus is immersed in an insulated metal jacket through which cold water (4° C.) is circulated or which may be filled with ice. When the apparatus is properly constructed the temperature variations in *A* are scarcely detectable with a Beckmann thermometer, hence they are unlikely to be greater than 0.005° C.

The device has been used to produce constant vapor pressures in a sorption apparatus by immersing in it a bulb containing the liquid in question. When unlimited supplies of cold water are available the device may be operated continuously at temperatures below that of the room for long periods of time without attention.

1. PIDGEON, L. M. AND EGERTON, A. C. J. Scientific Instruments, 8 : 268-269. 1931.

DIVISION OF CHEMISTRY,
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OTTAWA, CANADA.

L. M. PIDGEON.

RECEIVED NOVEMBER 17, 1933.

Improved Method for the Construction of Quartz Spirals

Quartz spirals for use in the McBain-Bakr sorption balance are generally made by winding a quartz fibre of suitable thickness on a carbon rod, using a small flame to soften the fibre at the appropriate point. Tapp (1) has described a system of gears which winds the spiral automatically. In all

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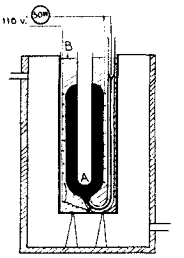

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cases, however, extreme care is required in handling the flame. If a flame hot enough to soften the fibre after it touches the rod is employed, there is danger of fusing the fibre before it is on the rod. On the other hand, if the flame is just sufficiently hot to soften the fibre in the air it is impossible to bend the fibre after it is on the rod owing to the heat loss by conductivity. The latter procedure has been the only safe one to employ, and it is apparent that the fibre must be bent to the correct curvature *before* it touches the rod. This requires very nice adjustment of the flame and the procedure is at best unsatisfactory.

The difficulty has been overcome by fluting the rod in such a way that the fibre touches at certain points only and the heat loss is thereby greatly reduced. The rod is placed in a lathe and longitudinal grooves are cut by racking the tool from side to side. A cross section of the rod after this treatment presents an appearance similar to a 10 or 12 pointed star. The fibre rides on the edges so formed and a large, comparatively cool, flame may be employed so that the fibre is heated on the rod for several turns back. Thus the fibre may be actually tightened while on the rod and there is much wider latitude in regard to the position of the flame. Some difficulty was experienced at first in removing the tightly wound spirals but this was obviated by turning a very slight taper on the rod before making the longitudinal grooves. With this modification the spiral slips off readily after being freed with a soft brush.

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FURTHER OBSERVATIONS ON THE POLLEN CONSTANCY OF BEES[1]

By W. H. Brittain[2] and Dorothy E. Newton[3]

Abstract

Further studies of the pollen constancy of hive bees and the wild bee visitors to apple blossoms, in continuation of those previously published (1, 2), are reported. Only hive bees and various *Andrena* spp. appeared in significant numbers in this study. The latter show a much lower degree of constancy than the former, confirming previous work. The wide difference between the complex of insect pollinators at Macdonald College and that of Kings County, Nova Scotia, is discussed. The highly polytrophic character of the common insect pollinators of the apple is emphasized.

Introduction

In previous papers (1, 2) the results of detailed studies on apple pollination, with regard to insect pollinators, have been discussed. The hive bee has, in comparison with solitary wild bees, certain advantages as an orchard pollinator. It has longer working hours and greater efficiency under unfavorable weather conditions; it increases rapidly and can be artificially distributed. It has been pointed out that, though the hive bee has the advantages mentioned, the small wild bees of the genera *Halictus* and *Andrena* have certain other advantages. In some fruit growing regions nesting places are abundant, and these are not necessarily reduced by modern agricultural practices, since roadsides, rough pastures, steep banks and similar situations may offer suitable sites. A minor point in favor of the solitary bees is their method of carrying pollen, which is more likely to result in cross-pollination of the flower than the more specialized mode of *Apis* or *Bremus*. As pointed out by Professor Cockerell (*in litt.*), *Halictus*, contrary to customary definition, has a scopa-like structure on the abdomen, and in some species, especially some of the Australian forms, it is quite well developed. The more specialized habits of the hive bee workers with respect to the division of labor were also considered to be an adverse factor in that the numbers of workers available for pollination were reduced.

[1] *Manuscript received December 13, 1933.*
Contribution from Macdonald College, Macdonald College Post Office, Que.
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One point in which superiority has been generally claimed for the hive bee has been its supposed greater constancy to its flower. In studies made in 1932 (2), however, no marked superiority of the hive bee over *Halictus* was demonstrated, though *Andrena* did appear to be noticeably less constant than either *Apis* or *Bremus*.

FIG. 1. *Aerial view of Macdonald College campus showing plant distribution, nesting sites and other ecological features. (Published by permission of Fairchild Aerial Surveys Company of Canada, Ltd.)*

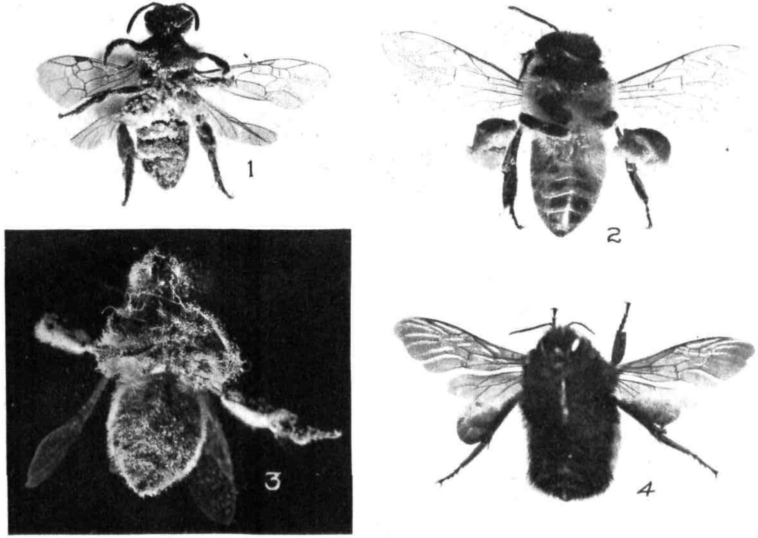
The present study, confined to Macdonald College, is a continuation of previous work on this subject. The main collections were made on apples, supplemented by smaller ones from various plants flowering at the same time or just subsequent to the apple.

Character of Locality Studied

Since a description has been given (1) of the various localities where previous studies were made it is necessary only to state that at Macdonald College there was available a 30-acre apple orchard, together with a profusion of flowering shrubs and herbs, whereas at all the observation points in Nova Scotia, apple greatly predominated. Fig. 1 indicates the nature of the territory where the present studies were made.

Insects Involved

It is of interest to note the species involved in apple pollination at Macdonald College, as compared with those concerned in this work in Kings



Ventral view of bees showing pollen carrying apparatus. 1. *Halictus coriaceus* Smith. 2. *Apis mellifica* L. 3. *Closer view of the preceding, showing pollen attached to body hairs.* 4. *Bremus fervidus* Fab.

County, Nova Scotia. In both areas the chief agents, in addition to the hive bee, consisted of solitary bees of the genera *Halictus* and *Andrena*. Diptera, chiefly Syrphidae, were of minor importance, and Bremidae comparatively scarce. A list of the Nova Scotia species has already been published (2), but further study has necessitated certain changes in the identification and synonomy of the species. Mr. C. E. Atwood, who has made a critical study of the Nova Scotia Andrenidae has revised the list and also determined the bees collected at Macdonald College in the present study. These lists are presented in Table I. In the list of species taken at Macdonald College, Diptera and Bremidae have been omitted as they played a very minor role.

TABLE I

LIST OF APPLE POLLINATORS IN NOVA SCOTIA AND AT MACDONALD COLLEGE

Nova Scotia		Macdonald College	
Halictus spp.	*Andrena* spp.	*Halictus* spp.	*Andrena* spp.
viridatus Lov. *(a)*	*carlini* Ckll.	*macoupinensis* Robt.	*annae* Ckll.
leucozonius (Schrank) *(b)*	*vicina* Sm.	*provancheri* D.T.	*carlini* Ckll.
zonulus Sm. *(c)*	*wilkella* Kby.	*sparsus* Robt.	*crataegi* Robt.
rubicundus (Christ.) *(d)*	*crataegi* Robt.	*viridatus* Lov.	*flavoclypeata* Sm. *(f)*
arcuatus Robt.	*rugosa* Robt.	*zonulus* Sm.	*forbesii* Robt.
cressonii Robt.	*milwaukeensis* Graen.		*mariae* var. *concolor* Robt.
provancheri D.T.	*bradleyi* Vier.		*miranda* Sm.
pilosus Sm.	*mariae* var. *concolor* Robt. *(e)*		*nasoni* Sm.
foxii Robt.	*miranda* Sm.		*lata* Vier.
pectoralis Sm.	*thaspii* Graen.		*persimulata* Vier.
coriaceus Sm.			*regularis* Mall.
			vicina Sm.
			wilkella Kby.

(a) H. smilacinae Robt. = *planatus* Lov. = *versans* Lov. *of former list (1). The writers' material agrees with authentically named specimens of viridatus. Should type material of smilacinae prove to be cospecific, the latter would have priority. (b) H. craterus of former list, in part. (c) H. craterus* Lov. *(d) H. lerouxii of list. (e) A. weedi of list. (f). A. bipunctata Cresson.*

Food Habits of Bees

The foregoing study opens up some interesting questions regarding the food habits of *Halictus* and *Andrena*, particularly the apple pollinators. Cockerell (*in litt.*) states that it may be laid down as a general rule that, *when a cultivated plant is not native in a region it will attract only the polytrophic species*. He states that in America the various cacti are noteworthy for their interesting bee visitors, largely *Diadasia* and *Lithurgus*, but, in South Africa, where *Opuntia* has overrun the country, it is useless to look for bees in the flowers.

It is of interest to examine the writers' host records in the light of the foregoing. Results of pollen analyses made at Macdonald College only are shown in Tables II and III. The following list shows the chief plant genera on which captures were made, and Tables IV and V indicate the bee species

taken on each. All available records from Kings County, Nova Scotia, and Macdonald College are included, but it should be noted that at Macdonald College little collecting was done except on apple. In Nova Scotia it was not possible to do any extensive collecting previous to apple bloom, and hence few records are available from the early spring flora. Later collecting was extensive though not exhaustive, and a more intensive search would doubtless yield additional species. Numerous species, represented in most cases by single specimens, have not been included because of uncertain identification.

LIST OF PLANT GENERA VISITED BY *Andrena* AND *Halictus*

1. *Achillea*
2. *Amelanchier*
3. *Amorpha*
4. *Apocynum*
5. *Aralia*
6. *Arctium*
7. *Aster*
8. *Brassica*
9. *Centaurea*
10. *Cerastium*
11. *Chrysanthemum*
12. *Cichorium*
13. *Cirsium*
14. *Cornus*
15. *Crataegus*
16. *Daucus*
17. *Diervilla*
18. *Epilobium*
19. *Fragaria*
20. *Hieracium*
21. *Kalmia*
22. *Ledum*
23. *Leontodon*
24. *Narcissus*
25. *Prunus*
26. *Pyrus malus**
27. *Pyrus* spp.*
28. *Raphanus*
29. *Rhododendron*
30. *Rhus*
31. *Rosa*
32. *Rubus*
33. *Salix*
34. *Solidago*
35. *Sonchus*
36. *Spergula*
37. *Stellaria*
38. *Syringa*
39. *Taraxacum*
40. *Trifolium*
41. *Vaccinium*
42. *Veronica*
43. *Viburnum*
44. *Philadelphus*

TABLE II

POLLEN ANALYSIS OF BEES AT MACDONALD COLLEGE—MAY 22-JUNE 3, 1933

No. examined	Bee species	Host	Pollen species
40	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus*
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Lonicera*
2	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Prunus*
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Spiraea*
16	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Taraxacum*
4	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Taraxacum* (1)
2	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Taraxacum* (2)
2	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Taraxacum* (3)
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Tulipa*
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Caragana; Taraxacum*
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Lonicera; Prunus*
2	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Spiraea; Taraxacum*
1	*Apis mellifica* L.	*Pyrus malus*	*Pyrus malus; Prunus; Taraxacum; Syringa*
2	*Apis mellifica* L.	*Syringa vulgaris*	*Syringa*
1	*Apis mellifica* L.	*Syringa vulgaris*	*Syringa; Lonicera*
1	*Apis mellifica* L.	*Syringa vulgaris*	*Syringa; Lonicera; Taraxacum*
2	*Apis mellifica* L.	*Berberis vulgaris*	*Berberis*
4	*Apis mellifica* L.	*Berberis vulgaris*	*Berberis; Caragana* (4)
16	*Apis mellifica* L.	*Lonicera*	*Lonicera*
1	*Apis mellifica* L.	*Lonicera*	*Lonicera* (5)
1	*Apis mellifica* L.	*Lonicera*	*Lonicera; Berberis*
1	*Apis mellifica* L.	*Lonicera*	*Lonicera; Pyrus malus*
1	*Apis mellifica* L.	*Lonicera*	*Lonicera; Viburnum*
3	*Apis mellifica* L.	*Caragana arborescens*	*Caragana*
2	*Apis mellifica* L.	*Caragana arborescens*	*Caragana; Lonicera*
1	*Apis mellifica* L.	*Caragana arborescens*	*Caranga; Viburnum*
1	*Apis mellifica* L.	*Caragana arborescens*	*Caragana; Berberis; Viburnum; Cornus*
1	*Apis mellifica* L	*Caragana arborescens*	*Caragana; Lonicera; Viburnum; Pyrus malus*
1	*Apis mellifica* L.	*Cornus paniculata*	*Cornus; Berberis*

**Material from Pyrus malus was separated from that collected on other species of Pyrus.*

TABLE II—*Continued*

POLLEN ANALYSIS OF BEES AT MACDONALD COLLEGE—MAY 22-JUNE 3, 1933

No. amined	Bee species	Host	Pollen species
1	*Apis mellifica* L.	*Cornus paniculata*	*Caragana; Prunus*
1	*Apis mellifica* L.	*Centaurea montana*	*Centaurea* (6)
1	*Andrena* sp.	*Pyrus malus*	*Pyrus malus; Taraxacum* (7)
1	*Andrena* sp.	*Pyrus malus*	*Pyrus malus; Taraxacum; Prunus*
1	*Andrena* sp.	*Pyrus malus*	*Pyrus malus; Taraxacum; Spiraea*
4	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus*
3	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Ribes*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Spiraea*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Taraxacum; Spiraea*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Taraxacum; Syringa*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Pyrus malus; Prunus; Ribes*
1	*Andrena annae* Ckll.	*Pyrus malus*	*Taraxacum*
1	*Andrena carlini* Ckll.	*Pyrus malus*	*Pyrus malus; Prunus*
1	*Andrena carlini* Ckll.	*Pyrus malus*	*Pyrus malus; Taraxacum*
7	*Andrena crataegi* Robt.	*Pyrus malus*	*Pyrus malus*
1	*Andrena crataegi* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum* (8)
1	*Andrena crataegi* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum; Caragana*
1	*Andrena crataegi* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum; Prunus*
1	*Andrena crataegi* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum; Ribes*
1	*Andrena crataegi* Robt.	*Cornus paniculata*	*Cornus; Caragana*
1	*Andrena crataegi* Robt.	*Lonicera tatarica*	*Spiraea; Caragana*
2	*Andrena flavoclypeata* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena flavoclypeata* Sm.	*Cornus paniculata*	*Pyrus malus*
3	*Andrena forbesii* Robt.	*Pyrus malus*	*Pyrus malus*
1	*Andrena forbesii* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena forbesii* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum* (9)
1	*Andrena mariae* var. *concolor* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena mariae* var. *concolor* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum; Prunus*
1	*Andrena miranda* Sm.	*Pyrus malus*	*Pyrus malus*
1	*Andrena miranda* Sm.	*Pyrus malus*	*Pyrus malus; Caragana*
1	*Andrena miranda* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena miranda* Sm.	*Pyrus malus*	*Pyrus malus; Viburnum*
1	*Andrena miranda* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum; Spiraea*
3	*Andrena nasoni* Robt.	*Pyrus malus*	*Pyrus malus*
2	*Andrena nasoni* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena nasoni* Robt.	*Pyrus malus*	*Pyrus malus; Taraxacum; Caragana* (10)
1	*Andrena lata* Vier.	*Pyrus malus*	*Pyrus malus*
1	*Andrena lata* Vier.	*Pyrus malus*	*Pyrus malus; Taraxacum; Caragana* (11)
1	*Andrena lata* Vier.	*Spiraea vanhouttei*	*Pyrus malus; Spiraea*
1	*Andrena persimulata* Vier.	*Cornus paniculata*	*Cornus*
1	*Andrena regularis* Mall.	*Pyrus malus*	*Pyrus malus; Taraxacum*
19	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus*
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Caragana*
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Prunus*
7	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum* (12)
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum* (13)
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum; Prunus*
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Taraxacum; Caragana*
1	*Andrena vicina* Sm.	*Pyrus malus*	*Pyrus malus; Prunus; Spiraea*
1	*Andrena vicina* Sm.	*Spiraea vanhouttei*	*Caragana; Berberis*
1	*Andrena vicina* Sm.	*Berberis vulgaris*	*Berberis; Caragana; Tulipa*
1	*Andrena vicina* Sm.	*Berberis vulgaris*	*Berberis; Caragana; Lonicera*
1	*Andrena wilkella* Kby.	*Berberis vulgaris*	*Lonicera; Narcissus*
1	*Andrena wilkella* Kby.	*Pyrus malus*	*Pyrus malus; Taraxacum; Viburnum*
1	*Andrena wilkella* Kby.	*Spiraea vanhouttei*	*Berberis; Caragana; Spiraea*

TABLE II—*Concluded*

POLLEN ANALYSIS OF BEES AT MACDONALD COLLEGE—MAY 22-JUNE 3, 1933

No. examined	Bee species	Host	Pollen species
1	*Andrena wilkella* Kby.	*Spiraea vanhouttei*	*Cornus; Spiraea; Pyrus malus* (14)
1	*Andrena wilkella* Kby.	*Caragana arborescens*	*Caragana; Narcissus; Pyrus malus*
1	*Halictus macoupinensis* Robt.	*Pyrus malus*	*Pyrus malus*
1	*Halictus provancheri* D.T.	*Pyrus malus*	*Pyrus malus*
1	*Halictus provancheri* D.T.	*Pyrus malus*	*Pyrus malus; Tulipa*
1	*Halictus provancheri* D.T.	*Cornus paniculata*	*Cornus; Taraxacum; Viburnum*
1	*Halictus* sp.	*Pyrus malus*	*Pyrus malus; Spiraea*
1	*Halictus viridatus* Lov.	*Cornus paniculata*	*Pyrus malus; Caragana; Syringa*
1	*Halictus viridatus* Lov.	*Pyrus malus*	*Pyrus malus*
1	*Halictus viridatus* Lov.	*Pyrus malus*	*Pyrus malus; Prunus*
1	*Halictus viridatus* Lov.	*Pyrus malus*	*Pyrus malus; Tulipa*
1	*Halictus viridatus* Lov.	*Pyrus malus*	*Pyrus malus; Taraxacum* (15)
2	*Halictus viridatus* Lov.	*Pyrus malus*	*Pyrus malus; Taraxacum*
1	*Halictus sonulus* Sm.	*Lonicera tatarica*	*Lonicera; Caragana*
1	(*craterus* Lov.)		
1	*Halictus sonulus* Sm. (*craterus* Lov.)	*Lonicera tatarica*	*Lonicera; Caragana; Spiraea; Tulipa*
1	*Halictus sonulus* Sm. (*craterus* Lov.)	*Lonicera tatarica*	*Lonicera*
1	*Bremus separatus* Cress.	*Pyrus malus*	*Pyrus malus; Syringa; Taraxacum*
1	*Bremus terricola*	*Pyrus malus*	*Pyrus malus* (16)
1	*Bremus terricola*	*Spiraea vanhouttei*	*Spiraea; Lonicera; Cornus*
1	*Colletes inaequalis*	*Pyrus malus*	*Pyrus malus*

(1) 1 grain Taraxacum only; (2) 2 grains Taraxacum only; (3) 3 grains Taraxacum only; (4) few Caragana; (5) also spores of Alternaria; (6) few grains only; (7) few Taraxacum only; (8) 1 grain Taraxacum only; (9) 1 grain Taraxacum only; (10) mostly Taraxacum; (11) 2 grains Taraxacum only; (12) 1 grain Taraxacum only; (13) 2 grains Taraxacum only; (14) heavy load of Pyrus; (15) 2 grains Taraxacum only; (16) 1 grain only.

The general results set forth in Table II can be studied best by reference to Table III.

TABLE III

SUMMARY OF PURE AND MIXED POLLEN LOADS CARRIED BY VARIOUS BEE SPECIES

Bee species	Pure loads		Mixed loads			
	No.	%	2 spp.	3 spp.	4 spp.	Total spp.
Apis mellifica	65	56.6	41	5	4	50
Andrena spp.	41	43.2	36	21	—	54
Halictus spp.	4	26.6	8	2	1	11
Bremus spp.	1	33.3	—	2	—	2

TABLE IV

THE HOST PLANTS OF *Andrena**

Insect	1	2	3	7	8	9	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31	32	33	34	37	38	39	40	41	43	44
A. algida Sm. *(b)*													×																					
A. annae Ckll																	×		×															
A. bipunctata Prov.		×							×										×								×							
A. bradleyi Vier. *(g)*																																×		
A. canadensis D.T.				×								×															×							
A. carlini Ckll.																		×	×						×	×	×					×		
A. carolina Vier. *(e)*															×				×	×														
A. ceanothi Vier.											×							×	×						×	×							×	
A. crataegi Robt. *(f)*		×							×		×						×	×	×					×	×									
A. flavoclypeata Sm.									×									×	×															
A. forbesii Robt.																		×								×								
A. frigida Sm.								×										×								×								
A. grandior Ckll.																		×						×	×									
A. hirticincta Prov.				×								×															×							
A. kalmiae Atwood														×	×																			
A. lata Vier.																		×	×						×									
A. mandibularis Robt.														×				×	×	×														
A. mariae var. *concolor* Robt.					×			×									×	×	×						×	×								
A. milwaukeensis Graen.								×											×						×									
A. miranda Sm.		×			×									×				×	×						×	×								
A. multiplicata (a)																																		
A. nasoni Robt. *(c)*		×																×																
A. nebecula Sm. *(b)*																											×							
A. persimulata Vier.								×																									×	
A. placida Sm. *(d)*																		×	×											×				
A. regularis Mall.				×															×						×				×			×		
A. robertsonii D.T. *(b)*																							×											
A. rugosa Robt. *(b)*																		×																
A. thaspii Graen.					×						×				×						×			×	×		×	×						
A. vagans Ckll. *(b)*													×																					
A. vicina Sm.		×															×	×								×								
A. wheeleri Graen.						×		×														×			×									
A. wilkella (Kby.)	×		×		×				×			×	×			×		×			×	×			×			×		×	×	×		×

NOTE.—*(a), no host records; (b), one host record only; (c), two host records only; (d), also found on Acer; (e), also found on Claytonia and Dentaria; (f), also found on Spiraea; (g), also found on Ribes.*

**The hosts are given a number corresponding to the accompanying list, and a positive record is marked* ×.

TABLE V

HOST PLANTS OF *Halictus*

Insect	1	2	3	5	6	7	8	9	12	13	15	16	17	18	19	20	21	23	24	25	26	29	30	31	32	33	34	35	36	37	39	40	41	42	44
H. arcuatus Robt.										X		X		X						X	X		X	X	X		X								
H. athabascensis Sandh. *(C.r.)*														X							X										X			X	
H. confusus Robt. *(a)*																																			
H. craterus Lov.	X																				X														
H. coriaceus Sm.			X				X				X		X								X	X			X		X								X
H. cressoni Robt.		X	X																	X	X				X								X		X
H. foxii Robt. *(s)*				X			X			X			X							X	X	X					X								
H. leucozonius (Shrank) *(r)*	X				X	X		X				X	X	X		X			X	X	X					X		X				X	X		X
H. lineatus Craw. *(a)*																																			
H. macoupinensis Robt.							X							X											X							X			
H. nymphearum Robt.																		X									X					X			
H. pectoralis Sm.	X					X	X		X	X			X	X		X		X						X	X				X	X					
H. pilosus Sm. *(r.a)*		X					X						X							X	X						X					X			
H. provancheri D.T. *(c)*							X							X	X			X		X	X						X								
H. quebecensis Craw.																	X			X	X														
H. rubicundus (Christ.)	X						X			X	X							X			X				X	X	X				X			X	
H. rufitarsus Zett.		X												X							X														
H. sparsus Robt.		X																	X	X					X		X				X				
H. viridatus Lov.				X		X	X		X				X	X				X			X	X				X	X				X		X		
H. sonulus Sm. *(s.v.)*	X						X		X	X				X		X		X			X			X			X	X	X			X			

NOTE.—*(a), no host records; (c), also taken on Cerastium; (c.r), also taken on Cornus; (r), also taken on Rudbekia; (r.a), also taken on Raphanus; (s), also taken on Spiraea; (s.v.), also taken on Senecio and Viburnum.*

Discussion of Results

An examination of Tables II and III does not alter the conclusion arrived at as a result of former studies (1, 2). *Halictus* and *Bremus* species, however, were taken in such small numbers that the results have no statistical value. *Andrena* spp. as a group, show a lower degree of constancy than *Apis mellifica* L., and less than that exhibited by *Halictus* in former studies, to which reference has been made.

Availability of any particular bloom again appears to be the main factor in determining the degree of constancy exhibited. It is only to be expected that where any particular plant species predominates the bees visiting the blossoms of that particular plant would show a high percentage of pure loads. It must be admitted, however, that these studies do not indicate as high a degree of flower constancy for the hive bee as has been claimed by some workers.

From an examination of Tables IV and V, in which the host records of the bees captured are summarized, it will be seen that species most commonly occurring on the apple are those that show the widest range of food plants. All species of both genera commonly occurring in the region appear among the captures from this host. The fact that a species is recorded from only a few hosts or from a single host does not necessarily indicate oligotrophy, since such species are those that appear to be rare in the region studied, and hence the probability of their being taken on any particular host is reduced. Doubtless, a more intensive survey and particularly a closer study of the early flora would produce results of interest.

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ON THE PREVALENCE OF PATHOGENIC FORMS OF *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM CULMORUM* IN THE SOIL OF WHEAT FIELDS AND ITS RELATION TO THE ROOT ROT PROBLEM[1]

BY G. B. SANFORD[2] AND W. C. BROADFOOT[3]

Abstract

A total of 227 isolates of *Helminthosporium sativum* and 286 of *Fusarium* sp. (*culmorum* type) were obtained from the diseased crown tissue of wheat stubble in five fields located in the black soil belt of central Alberta, and an attempt was made to determine their relative virulence on wheat seedlings and on mature plants. The experiment was carried out under greenhouse conditions, with a range of soil temperature. Sterilized, artificially infested soil in open pot culture was used. The results indicated that the *Helminthosporium* isolates were as a rule moderately to weakly pathogenic, and that most of the *Fusarium* isolates were only weakly pathogenic to wheat plants in the seedling stage. Some isolates of each pathogen exhibited extreme virulence, but judging from the results on seedling plants, virulent strains were rather rare in the fields studied. On mature plants both fungi showed about equal degrees of virulence, which was on the whole weak, and the results were not considered significant for the purpose of the study. More isolates of the greater degrees of virulence were obtained from certain fields than from others. In view of the great susceptibility of seedling plants in sterilized re-infested soil and the variable results, presumably caused by association effects of contaminants of the soil in open pot culture, it was concluded that the object of the study could not be attained by means of data based on the seedling stage, or by the technique employed. The possibility of significant results being secured in sterilized re-infested soil, protected from contamination, and based only on mature plants, is discussed in relation to the root rot problem.

Introduction

It is well known that there is much variability in virulence among isolates of either *Helminthosporium sativum* P.K. and B., or *Fusarium culmorum* Smith, both of which are root rotting pathogens of cereals, and that the phenomenon is a type of physiologic specialization common among many fungi.

Stakman (15), in discussing "Racial specialization in plant disease fungi", dealt with its significance in breeding for resistance, and reviewed the contributions of many workers on this subject. Stevens (17) and Christensen (5) and others have given evidence of the diversity of *H. sativum*, and Brown (4) and others, of the diversity in the genus *Fusarium*. Craigie (7) showed how physiologic forms of *Puccinia graminis tritici* may originate by hybridization on *Berberis vulgaris*. Hanna (9), Stakman *et al.* (16) and Christensen (6) have demonstrated that many physiologic forms of *Ustilago zeae*, which differ in virulence, exist and that they may originate by hybridization and mutation. The interesting treatise of Brierley (1) deals theoretically with the origin of racial diversity among fungi and bacteria. Dickinson

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(8) concluded that saltant strains of *F. fructigenum* are the result of mutations, while Hansen and Smith (10), from work on *Botrytis cinerea* Pers., maintain that the variable forms of Fungi Imperfecti may owe their instability, not to mutation, but to nuclear heterogeniety (heterocaryosis) induced by anastamosis of hyphae and unequal cell divisions.

By whatever method diverse forms may occur in *H. sativum* and *F. culmorum*, the fact that they are numerous in nature probably affects greatly the problem of synthesizing varieties resistant to the root diseases of cereals which they cause. The work of Broadfoot (3) indicated that both fungi are extremely common in the crown tissue of wheat in average field culture, and that a number of crop sequences appeared to have no perceptible effect on their relative prevalence as suggested by isolations. But the immediate problem in a plant breeding program appears to be concerned, not so much with the prevalence of these organisms, as with the degree of virulence most common in the average field. Apparently no information is available as to whether certain crops make any perceptible difference in the ratio of the more virulent forms to those less virulent. If virulent forms are relatively scarce in our soils, and moderate pathogenicity the common thing, it would appear that it would be advisable to obtain first the necessary resistance to withstand the moderate degree of virulence common under field conditions. Particularly is this true when our wheat varieties do not possess marked resistance. Therefore, the object of this study was to obtain a general picture of the degree of virulence which exists among isolates* of *H. sativum* and *F. culmorum* in wheat fields in Alberta.

Materials and Methods

Choice of fields. In accordance with the objects of this study, each year from 1928 to 1932 inclusive, one field of wheat, suffering from *Helminthosporium* root rot and *Fusarium* root rot, was located at some point in the black soil belt of central Alberta. The soil reaction of these fields, according to the report of Wyatt and Newton (19), would be approximately pH 6.2. Fields suffering from typical *Ophiobolus* root rot (take-all) were, as far as possible, avoided, and isolations were not made from plants known to be suffering from this root rot. However, the writers' opinion, which is supported by the work of Robertson (12), is that plants may not indicate the typical take-all symptoms, even though the major injury is caused by *Ophiobolus graminis* and the tissue yields either or both *H. sativum* and *F. culmorum*. Stubble was pulled from five different fields at harvest time. The location of each field is indicated in Table I. The samples of stubble from which isolations were made were taken at random from an area of about four acres. About 80 separate liberal samples of the stubble were pulled from as many equidistant points in each field and numbered.

Obtaining isolates. From each composite sample of stubble the crown and the main tiller base of several plants showing signs of crown infection were

* *Used in this paper as a noun in the sense of 'isolations' and without regard to possible racial differences.*

taken. This portion was surface disinfected for two minutes in mercuric chloride solution (1:1000) and incubated on solid potato sucrose agar. Subsequently, pure cultures of *H. sativum* and *F. culmorum** were obtained, from which single spore isolates were chosen for the pathogenicity test to be made. The total number of isolates of *H. sativum* and *F. culmorum* thus obtained was 513, made up of 227 of the former and 286 of the latter. As these were tested at various temperatures, both in the seedling and maturity series, the total number of tests of *F. culmorum* in the seedling stage was 668, while for the mature series it was 291. The corresponding figures for *H. sativum* are 487 and 133.

Pathogenicity tests. These were made in open pot culture in sterilized artificially re-infested soil, under greenhouse conditions during the late fall, winter and early spring months. The reduced daylight during this period was supplemented by electric illumination. In each case the isolations were tested for damage to the seedling stage, and in all but two cases for that to the mature plants. A range of soil temperature, the lowest being 13° C. and the highest 21° C., was employed. Each isolate chosen for test was increased on ground sterilized oat hulls and aged for 30 days, after which 10 gm. of it was scattered at seed level in each six-inch pot, and covered. For the seedling series, 15 grains of Marquis wheat were planted in each of the four replicate pots used to test each isolate, and for the mature series, seven grains. The number of control pots in each temperature series was approximately one-tenth of the test pots involved. The temperature used, and the number of isolates of each fungus tested at each temperature, in both series mentioned, are shown in Table I.

Estimating virulence. The degree of injury of each plant was estimated numerically, taking into consideration the percentage of plants which did not emerge, the condition of the crown, the mesocotyl, the primary root system, and the height of the plant. This rating was translated into percentage infection, and the isolates arranged in virulence classes, as given in Table I. The condition of the coleoptile was disregarded as, in the writers' opinion, it is early subject to discoloration by ordinary saprophytes, even in the soil of control pots.

Results

The summarized data, which are given in Table I, will for convenience be elaborated for each field. Some of the data are presented in chart form in Fig. 1.

Seedling Series, F. culmorum

Taking first the seedling series of the Camrose field, all of the 63 isolates tested at 16° C. fell into the virulence class below 10%; 93.8% of those tested at 18° C., and 93.1% of those tested at 17° C. fell into the same class. None exceeded the 20% class.

**Wherever F. culmorum is mentioned subsequently in this paper, Fusarium of the culmorum type is intended.*

TABLE I

THE VIRULENCE OF RANDOM ISOLATES OF *Fusarium* SP* AND *Helminthosporium sativum* FROM WHEAT STUBBLE OF FIVE DIFFERENT FIELDS, EXPRESSED ON (I) THE SEEDLING STAGE, AND (II) MATURE WHEAT PLANTS. THE PERCENTAGE OF ISOLATES IN THE VARIOUS VIRULENCE CLASSES IN EACH TEST ARE INDICATED FOR EACH PATHOGEN

Series	Fields	Temp., °C.		*Fusarium* sp.										No. Iso-lates	*H. sativum*										No. Iso-lates
				Virulence classes, %											Virulence classes, %										
		Air	Soil	0+	10+	20+	30+	40+	50+	60+	70+	80+	90+		0+	10+	20+	30+	40+	50+	60+	70+	80+	90+	
I. Seedling	A		18	93.8	6.2									64	81.8	18.2									11
			16	100										63	100										11
			17	93.1	6.9									58	90.0	10.0									10
	B	21	17	97.0				3.0						33	25.0	30.0	17.5	15.0	5.0	2.5	5.0				40
	C	19	17	55.2	30.6	4.2	2.0	2.0	2.0	2.0				49	2.6	15.8	15.8	47.4	7.9	7.9	2.6				38
		23	20	41.3	58.7									46	2.6	5.3	28.9	34.2	23.7	5.3					38
	D	24	21	12.3	46.5	30.1	1.4	1.4	1.4		1.4		5.5	73		1.6	4.8	11.1	14.3	20.6	25.4	17.5	4.7		63
		16	13	19.4	62.5	6.9	2.8				1.4	2.8	4.2	72	3.1	20.3	31.3	15.6	12.5	17.2					64
		21	18	9.7	65.4	13.9	4.0	1.4	1.4	1.4	1.4	1.4		72	1.6	4.7	18.8	15.6	12.5	14.0	14.0	9.4	4.7	4.7	64
		22	19	25.0	58.8	8.8	1.5	2.9		1.5		1.5		68	3.1	7.8	4.6	25.0	31.3	6.3	9.4	7.8	4.7		64
	E	19	17	53.7	28.3	9.0	1.5	4.5		3.0				67	24.3	25.7	14.9	18.9	13.5	2.7					74
Summary				51.3	35.2	7.7	1.4	1.4	0.4	0.8	0.4	0.4	1.0		13.2	15.2	15.0	19.3	14.2	9.2	7.0	4.5	1.8	0.6	
II. Mature	A		18	10.9	35.9	40.6	10.9	1.7						64	54.5	36.4		9.1							11
			16	15.9	41.3	26.9	15.9							63	36.4	63.6									11
			17	22.4	55.2	20.7	1.7							58	45.5	54.5									11
	B	21	17	33.3	54.6	9.1				3.0				33	75.0	25.0									40
	D	18	15	20.6	67.1	12.3								73	1.7	31.6	33.3	25.0	6.7	1.7					60
Summary				19.2	50.9	23.0	6.3	0.3		0.3					34.6	34.6	15.0	12.0	3.0	0.8					

*Of the *culmorum* type. †A, Camrose; B, Bittern Lake; C, Westlock; D, Fort Saskatchewan; E, Edmonton.

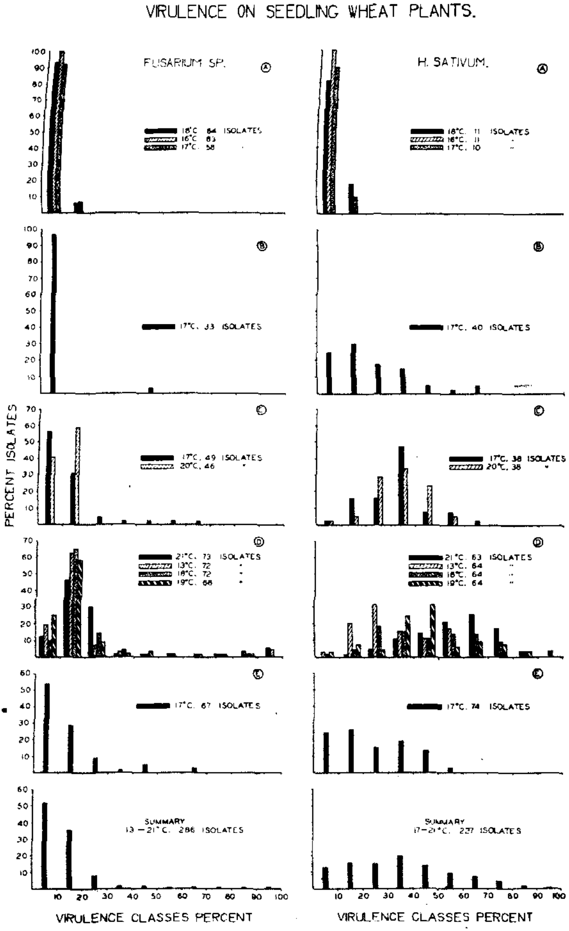

FIG. 1. *The prevalence in percentage of pathogenic isolates of Fusarium (culmorum type) and Helminthosporium sativum, as obtained at random from the wheat stubble of five fields. The pathogenicity was read on seedling wheat plants. A, B, C, D, and E indicate the location of each field (see Table I).*

Of the 33 isolates from the Bittern Lake field tested at 17° C., 97% were in the class below 10%, and the balance were in the class from 40 to 49%.

Of the 49 isolates from the Westlock field, tested at 17° C., slightly over 55% of them fell into the lowest virulence class, 30.6% of them into the next higher class, 4.2% into the next class, and 2% into each of the classes 30+, 40+, 50+ and 60+. In the test made at 20° C., 41.3% were in the lowest class, and the remainder in the next class.

Fort Saskatchewan isolates provided the most interesting results, because a much larger proportion of them fell into higher virulence classes than was the case with those from other fields. For example, we find in some instances approximately 1.4% of the isolates in the class above 60%, and two instances, namely 5.5% and 4.2% of the 72 to 73 isolates in the class above 90%. Apparently the differences in soil temperature did not affect greatly the distribution of the isolates within the virulence classes, at least as much as one might expect in view of previous work. Although the writers are unable to offer an explanation for the greater virulence shown by the isolates from this field, it may be mentioned that the wheat stubble, from which these isolates were obtained, followed a crop of barley, whereas those from other fields were obtained from the stubble of the third consecutive crop of wheat.

Results from the 67 isolates from the Edmonton field are on the whole comparable to those obtained from the Westlock field, 82% of them falling into the virulence class below 20%. None were as virulent as several isolates from the Fort Saskatchewan field.

Thus, considering all isolates from all five fields, 51.3% fell into the lowest class, 35.2% into the next higher class, 7.7% into the 20+ class, and 1.4% into the 30+, and 40+ classes, respectively, and less than 1% into those between 40+ and 90+ virulence, and only 1% into the highest class. Perhaps most obvious is the rarity (except in the peculiar case of the Fort Saskatchewan field) of virulent strains from the fields, the great bulk of the isolates being only slightly pathogenic to non-pathogenic on wheat in the very susceptible seedling stage.

Mature Series, F. culmorum

There is a striking contrast between the virulence of *F. culmorum* as indicated at the mature stage, and that at the seedling stage. A possible reason for this will be given later in the discussion of results. In most instances a smaller percentage of the isolates fell into the lowest virulence class and, in general, more into the next higher class, than was the case in the seedling series. Practically all of them fell into virulence classes below 40%. From 36 to 67% of the isolates from the three fields tested at various temperatures fell into the 10 to 19 virulence class, with a larger proportion of them indicating approximately 12%. Of the 33 isolates from the Bittern Lake field, and the 73 isolates from the Fort Saskatchewan field, 9.1 and 12.3% were in the 20 to 29% class, while of those from the Camrose field (isolations from Westlock and Edmonton fields were not tested in mature series) 20.7 to 40.6% were in this class.

Although in the three tests made at 16, 17 and 18° C., the same isolates were used, there was considerable variation in results. While it is possible that some of this irregularity may be accounted for by the size of class used there were other factors, the importance of which will be mentioned later.

TABLE II

TYPICAL EXAMPLES OF THE VARIABILITY IN VIRULENCE DURING FOUR TESTS ON SEEDLING WHEAT PLANTS OF THE SAME ISOLATES OF *Fusarium* SP.* AND *Helminthosporium sativum*

Isolates	1st test		2nd test		3rd test		4th test		Average	
	Plants**	R†	Plants	R	Plants	R	Plants	R	Plants	R
Fusarium sp.										
P-10	0.7	96	3.5	82	4.7	69	10.2	31	4.7	69
13	0.7	92	1.2	92	1.7	88	2.2	85	1.4	90
R- 2	13.5	13	0.5	93	14.0	20	12.5	17	10.1	37
7	4.2	76	6.5	83	14.0	16	11.7	23	9.1	49
19	0.5	96	1.7	91	11.0	30	13.2	11	6.6	57
H. sativum										
14-13	7.0	69	8.5	56	5.2	97	12.2	34	8.2	64
15- 1	7.5	66	11.2	50	7.0	94	10.5	39	9.0	62
3	3.0	86	10.2	58	8.0	59	9.0	46	7.5	62
16-17	4.5	78	9.7	50	7.0	67	7.2	58	7.1	63
17-11	2.7	87	8.7	59	6.5	71	3.5	80	5.3	74
Controls	13.7		13.7		14.0		13.6		13.8	
Air temp., °C.	24		16		21		22			
Soil temp., °C.	21		13		18		19			

*Of the culmorum type. **Average number of plants per pot from 15 grains. †Infection rating, percentage.

Examples of this type are given in Table II, where it will be observed that the infection rating for the same isolates varied greatly in the four separate tests made. For instance, *F. culmorum*, Culture *P*-10, was quite pathogenic in the first, second and third tests, but not in the fourth one; Culture *R*-2 was pathogenic in the second test, but not in the others; Cultures *R*-7 and *R*-19 were pathogenic in Tests 1 and 2, but not in the others; whereas Culture *P*-13 was pathogenic in all four experiments. Similarly, with *H. sativum*, although the variation was not so great, Culture 17-11 was quite pathogenic in all experiments, but Cultures 14-13 and 15-1 were quite pathogenic in Tests 1 and 3, and not in 2 and 4, whereas Cultures 15-3 and 16-17 were quite pathogenic in Test 1, but not as pathogenic in the other three trials. This variability is also clearly indicated by the number of surviving plants. Undoubtedly, such results cannot be explained on the basis of the slightly different soil temperatures of the four tests. Similar variability was evident for many other cultures during the five years of study.

However, the data given in Table I suggest that in the mature series practically all the isolates fell into the virulence classes below 30%, although a number of them were in higher classes in the seedling stage. It also indicates, as

ar as can be determined by the technique used, that the degree of virulence of *F. culmorum*, as it exists in the soil of wheat fields, is on the whole slight. Apparently very virulent strains are not prevalent, or they were not indicated by the method.

Seedling Series, H. sativum

Only 11 isolates of *H. sativum* were obtained from the Camrose field, the ssue from the crowns being almost wholly species of *Fusarium*. However, ll of these, subjected to three different tests at temperatures 16, 17 and 18° C., espectively, exhibited only slight virulence, and none exceeded 10%. In ne case all were in the lowest class, in another, 90%, in a third, approximately 2%.

Of the 40 isolates from the Bittern Lake field, tested at 17° C., 25% were n the class below 10%, 30% in the 10+ class, 17.5%, 15% and 5% in classes 20+, 30+ and 40+, respectively, and of the remainder, 2.5 and 5% in the 50+ and 60+ classes.

The results from the 38 isolates from the Westlock field, tested at 17° C., were on the whole fairly comparable with those from the 40 isolates obtained rom the Bittern Lake field, except that there was a tendency for greater virulence, as indicated by the fact that only 2.6% of them fell into the lowest class, 47.4% into the 30+ class, and 15.8% into each of the classes between. None exceeded the 60+ class. Similarly, at 20° C. the larger proportion of hem, namely 28.9, 34.2 and 23.7% were in the 20+, 30+ and 40+ classes, espectively, while of the remainder 2.6, 5.3 and 5.3% were in the 0+, 0+ and 50+ classes. It will be observed that at 20° C. there was a greater hift toward the higher classes than at 17° C. This tendency is also apparent with the 64 isolates from the Fort Saskatchewan field, where he results at 18, 19 and 21° C. may be compared to those from the test made at 13° C. However, it is obvious that the difference is not as great as might be expected, in view of the prevailing idea on the differential effects of temperature. Of particular interest is the tendency for a greater virulence of the solates from the Fort Saskatchewan field than of those from the other four ields, and in this respect comparable to the results from the *Fusarium* isolates rom the same stubble.

Considering the results from all five fields, it is clear that a much larger proportion of the isolates of *H. sativum*, as obtained at random from the field stubble, were much more pathogenic to wheat in the seedling stage than were he *F. culmorum* isolates. Approximately 85% of the *F. culmorum* isolates ndicated virulence below 20%, while about 30% of the *H. sativum* isolates ell into this class, the others into classes up to 90%, and the major portion of them into classes between 20 and 50%.

Mature Series, H. sativum

The virulence of the 11 isolates from the Camrose field, tested at 16, 17 nd 18° C., indicated little damage over that occurring in the seedling series. Seventy five per cent of the 40 isolates from the Bittern Lake field, tested

at 17° C., fell into the lowest class, and the remainder into the 10 to 19% class, thus indicating much less damage than occurred to plants in the seedling stage. The 60 isolates from the Fort Saskatchewan field, tested at 15° C., also indicated a lower virulence than they produced on the seedling stage at 13° C.; 90% of them being in the classes from 10 to 39%, and of the balance 6.7% were in the 40 to 50% class, and 1.7 in the 50+ and lowest classes, respectively.

Discussion

In general, the data presented indicate that moderate to weak virulence was the rule with the *Helminthosporium* isolates in the seedling series, and that the *Fusarium* isolates were, on the whole, only weakly pathogenic. A few isolates of both fungi exhibited marked virulence and, according to the results, these were more common in certain fields than in others but, in any case, their occurrence was relatively rare. On mature plants the isolates of both fungi showed about equal degrees of pathogenicity, which was weak or apparently absent, particularly in the case of the *Fusarium* isolates. If the foregoing results can be accepted as reliable, the study was successful in demonstrating that moderate to weak pathogenicity is the rule in the average field, and that very virulent strains are relatively rare, and consequently the plant breeding problem is, for the moment, simplified in proportion.

Thus comment appears to centre on whether the method used was suitable for the purpose indicated. Admittedly, the general technique employed in testing the isolates in open pot culture in sterilized soil was that which has been used by many workers in the past, and the one on which most of the published results on the root rotting pathogens *H. sativum*, *F. culmorum* and *O. graminis* have been based. As mentioned earlier, there were four pot cultures for testing each isolate each time at the various temperatures. Thus, in testing the *Fusarium* isolates there were 2672 test pots involved in the seedling series, and 1164 pots in the mature series, and for the *Helminthosporium* isolates the corresponding numbers were 1948 and 532. However, in spite of the liberal replications, the great variability which occurred among separate tests, among replicate pots, and among the plants within a given pot, and the fact that the results from the mature series were roughly opposite to those obtained on the seedling plants, throws much doubt on the significance of the data or its value for the purpose sought. As a matter of fact, in one test (seedling series) an isolate might indicate weak pathogenicity, and in another, under apparently similar conditions, be definitely virulent, and *vice versa* (Table II).

Possibly some of the variability indicated might be accounted for by genetic changes in the isolates, but, with open pot culture methods, using sterilized soil, the results obtained could probably not have been much different. The pathogen in sterilized soil would at first exert its full effect on the very susceptible seedling plants, but in a short time contaminants (fungi and bacteria) or combinations of these, which may be antagonistic to the isolates being tested, quickly occupy the sterilized soil to the detriment of the pathogen

under test. The writers (13) have demonstrated this in connection with *O. graminis*, and have found by experiment that it also applies to *H. sativum* and *F. culmorum*. Henry (11) has also demonstrated the phenomenon as it concerns the last mentioned pathogens. Further, Broadfoot (2) has shown how quickly the virulence of *O. graminis* may be vitiated in sterilized soil in open pot culture. For example, its virulence on wheat seedlings dropped from 65 to 48, 41, 20, 25, and 13%, when planted 10, 20, 30, 40 and 50 days, respectively, after the inoculum was added to the originally sterilized soil. It appeared practically impotent at the end of 120 days. Thus, in view of the differences mentioned, it is doubtful whether the data obtained in the mature series of this study are significant. In regard to those obtained from the seedling series, it is necessary to consider the great susceptibility of these plants, especially in sterilized soil, where even weak pathogens may be effective, and often sufficiently so to prevent the demonstration of definite pathogenic differences among the isolates.

To avoid the difficulties mentioned, it would seem necessary that the test be made in sterilized re-infested soil protected from outside contamination, and that results be based only on the reaction of mature plants. The inoculum of the isolate would then be added during the post-seedling stage. The question is also raised whether plants, after the post-seedling stage, become more or less susceptible toward maturity and, as well, the relation of resistance to vigor, as affected by light intensity, moisture, temperature, nutrients, and the pentosan content of the plants, all of which are not well understood at present. Tyner (18) found that wheat plants, grown under normal illumination, had a definitely higher pentosan content per unit mass than when the light was subnormal, and that increased pentosan content appeared to be associated with increased vigor and greater resistance to root rot caused by *O. graminis*.

Thus, with sufficient information, standard experimental conditions could be evolved and used in an attempt not only to produce a general picture of the virulence of root rotting pathogens in the average wheat field, but possibly to determine the degree of natural resistance which varieties and hybrids may possess. Obviously the performance in the field of a variety which may indicate resistance under laboratory conditions must, in the final analysis, be ascertained.

Testing for natural resistance of varieties in the field in infested soil would theoretically give positive results, provided a sufficient degree of resistance existed. However, in view of the possible confusing effect of diverse forms of each pathogen, and the well recognized effect of extremely variable environmental factors in the field, and the apparently indefinite resistance which our present varieties possess, the field method would seem to have very great limitations. At least, results at this laboratory (14) from testing in the field over 100 varieties and hybrids for resistance to these pathogens during the past six years would seem to support this contention. Moreover, in western Canada, it is in the damage to the post-seedling stage of wheat

plants that the main problem lies. In the writers' opinion, seedling blight, although important in certain seasons and cases would easily occupy a secondary position. Serious root rot damage by these fungi does not begin to manifest itself until late in June, unless under drought conditions, when it is too late for natural compensating factors to operate effectively.

Finally, the urgent need for better information must be admitted, for it is doubtful whether we have an adequate conception of the root rot problem as it is affected by the prevalence of pathogenic forms in the field, or a definite indication of the natural resistance which our varieties of cereals may or may not possess.

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BACTERIOLOGICAL STUDIES ON THE RED DISCOLORATION OF SALTED HIDES[1]

By A. G. Lochhead[2]

Abstract

A study was made of organisms concerned with the red discoloration of salted hides, also termed "red heat", which defect may occasion loss in the leather industry through spotting and weakening of the fibre. Red halophilic sarcinae were isolated from Argentine hide. From Canadian hides showing red discoloration, two species of pleomorphic rods were isolated as active agents. One of these, occurring on salted cowhides, was found to be similar to *Serratia salinaria* (Harrison and Kennedy) Bergey *et al.*, a source of reddening of cured codfish in eastern Canada. The other organism causing discoloration, isolated from buffalo hide, was regarded as a new species and designated *Serratia cutirubra* n.sp. Both of these halophilic organisms, owing to their proteolytic action, are considered capable of greater damage to hides than the red sarcinal types which are non-liquefying, and which may also be present on Canadian hides. Non-chromogenic halophilic bacteria were also isolated from discolored hides. These develop at a lower salt concentration range than the red organisms and are probably less active in causing injury to fibre in well salted hides.

Introduction

In September 1932 the attention of the writer was called by Dr. W. E. Graham of the National Research Council to the question of the reddish discoloration frequently appearing on salted hides. This question had been raised by the Associate Committee on Leather of the National Research Council and was under consideration in its general aspects by Dr. Graham. This discoloration, commonly referred to in the leather trade as "red heat", becomes evident on the flesh side of the hide during storage and represents a defect which may occasion considerable loss to the industry through spotting and weakening of the fibre.

The appearance of the discoloration suggested a possible analogy to somewhat similar defects occurring on other salted products, notably codfish, caused by salt tolerant bacteria, types of which might be expected to be active on salted skins. Samples of discolored hides were placed at the disposal of the writer by Dr. Graham, and the present report summarizes the work on the isolation and study of the causal and associated organisms.

Historical

A number of studies have already been reported dealing with the relation of micro-organisms to the appearance of red discoloration on various salted animal products, particularly fish, though occasional references to work with other products, such as sausage casings and hides, may be found. The various organisms indicated as causal agents represent a variety of types including micrococci, sarcinae, spore-forming and non spore-forming rods, spirochetes, algae and yeasts. While in many cases the organisms concerned were prob-

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ably the same, an accurate comparison is difficult or impossible owing to the incompleteness of many of the descriptions. Furthermore, in some of the earlier work especially, there are omitted many essential details, such as the media employed, the effect of salt concentration on the organism, and in some cases, the critical experiment of producing the reddening with the organism said to be the cause.

A detailed review of the work reported dealing with the reddening of fish would appear out of place. For this the reader is referred to such publications as those of Kellerman (9), Harrison and Kennedy (8), Cloake (5), and Hanzawa and Takeda (7). It is of interest, however, that there is almost complete unanimity in ascribing the source of infection to the various salts used for curing. The probability of similar sources of organisms causing discoloration of hides suggests a close relation of the reddening of these two salted products.

The first reported work on the red discoloration of salted hides appears to be that of Becker (2) who cultivated from reddened areas a strictly aerobic micrococcus, 1.1–1.5 μ in diameter, isolating in addition a species of torula, with cells approximately 6–7.5 μ in diameter. Since neither the micrococcus nor the torula showed any power of gelatin liquefaction, the author regarded the discoloration as harmless. Discoloration with more deleterious effects has been reported anonymously (1). Red spots noted in raw salted calfskins, which were found to be coextensive with damaged spots in the grain after finishing, were stated to be due to aerobic, proteolytic bacteria. On the other hand, in a report issued by "Frigorifico", according to Stather and Liebscher (20) the red discoloration on the flesh side of raw hides is caused by strongly aerobic, halophilic bacteria or molds which develop superficially, causing no material damage.

In 1929 Stather and Liebscher (20) reported the first of a series of investigations on the pink to brick red discoloration occurring on the flesh side of raw hides which they consider due to micro-organisms. The discoloration is regarded as a definite defect, resulting in a weakening of the hides, histological and chemical analyses indicating a definite loss of hide substance. Later the same authors (21), reporting their bacteriological studies, describe seven organisms isolated from reddened areas which they classify as *Sarcina lutea*, *Sarcina aurantiaca*, *Micrococcus tetragenus*, *Micrococcus roseus*, *Bacillus subtilis*, and two organisms regarded as *Proteus* and *Actinomyces* types respectively. Of these the last two organisms together with *S. aurantiaca* and *M. roseus* were chromogenic in various shades of red on artificial media. Of these, two were suppressed by a concentration of 8%, the others by 16% sodium chloride which raises doubt whether such types, widely distributed in dirt and dust, develop sufficiently to cause discoloration on the surface of salted hides. No examination for strict halophiles appears to have been made.

Investigating "red heat" of salted hides, Lloyd, Marriott and Robertson (12) paid special attention to halophilic organisms occurring in curing salts. With media of 16 to 30% salt concentration, strains of red and yellow sarcinae were isolated. Both types were obligate aerobes, strongly halophilic, grew

best at 37° C. and at 100% humidity. Gelatin was liquefied by the yellow, though not by the red organisms, consequently the latter were considered less likely to damage the hide fibres. The authors point out that on salted hides a mixed flora of halophiles and other organisms exists and suggest that the actual damage may be due to associative action.

Referring to the above work, Stather (19) suggests that the halophilic organisms causing "red heat" are not essentially different from the types isolated by himself and Liebscher from "red discoloration". The organisms from the latter were trained to survive on 20% salt, while cultures of halophiles of the English workers were said to have been trained to withstand low concentrations. The question of the relative ability of the organisms to produce the effect on salted hides was apparently not considered. Robertson (17), in considering whether halophilic organisms alone are responsible for red growths on hides, or whether ordinary red chromogens of dust and dirt also play a part, reports an investigation of the capacity for growth and survival at different salt concentrations of the two types. The red halophiles were found to flourish best at high salt concentrations, failing to develop with concentrations below 6 to 10%. On the other hand the organisms of dirt and putrefaction, including those described by Stather and Liebscher (21), failed to multiply with more than 8% salt, though some survived for various periods in the higher concentrations. Robertson believes that marine curing salts are the source of the halophiles causing discoloration, though in a later paper (18) she indicates the possibility that chromogenic dust and dirt organisms may play a minor part. A study of the effect of various antiseptics showed boric acid and borax to be useless for inhibiting halophilic growth, while sodium carbonate and sodium bisulphite produced ill effects on the hide fibre. Naphthalene, while inhibiting the bacteria for a few weeks without damaging the hide, lost its effect after this period, presumably owing to volatilization. Sodium fluoride, 0.5%, added to the curing salt, was stated to be the most satisfactory antiseptic tried.

A red discoloration of salted hides due to an obligate halophilic coccus is reported by Blankow (3). In line with the findings of Lloyd and coworkers are his observations that marine curing salts are the source of infection. Further evidence that hide curing salts are the source of infection is given by Stuart, Frey and James (22). In 34 out of 35 samples of crude solar salts and in 25 out of 29 open pan evaporated salts red chromogens, capable of causing reddening on calfskin, were found. On the other hand all kiln dried solar salts, all vacuum pan evaporated salts and all mined or rock salts examined were found to be free of such organisms.

Experimental

Methods

For the isolation of the halophilic organisms studied, three special media were used, employed with varying concentrations of salt.

Medium A:—beef extract, 3 gm.; yeast extract, 3 gm.; peptone, 10 gm.; soluble starch, 10 gm.; agar, 15 gm.; salt and distilled water to make 1000 cc.

Medium B:—½ lb. minced codfish steamed with one litre water for one hour and strained through several layers of cheese cloth; peptone, 1.0 gm.; glycerine, 5 gm.; agar, 15 gm.; salt and water to make 1000 cc.

Medium C:—to prepare 1 litre, 500 cc. fresh skimmilk, 15 gm. agar in 200 cc. water and salt, depending on the sodium chloride concentration desired, are sterilized separately. The hot ingredients are mixed in one flask, by adding the milk to the salt and this mixture in turn to the agar. Additional hot water is added gradually with shaking to make a final volume of 1000 cc. When the salt is dissolved the hot medium is poured into Petri dishes (or tubes for slanting) and when hard is ready for use.

The more detailed cultural and physiological tests on the organisms were carried out on various substrates at a favorable salt concentration, the effect of sodium chloride having been determined for each organism, using the most suitable basic medium. Observations were continued for three to four weeks and longer in the case of gelatin liquefaction. This latter determination presents some difficulty owing to the tendency of salt to prevent solidification. By using 25% gelatin, however, a medium containing 20% sodium chloride was prepared, which though not as firm as ordinary gelatin media, was satisfactory for determining liquefaction when used as slants with surface inoculation by the loop method.

For microscopical examination and slide preparation the organisms were suspended in a salt solution the strength of which corresponded approximately to that of the medium. In the case of the non-chromogens and the red sarcinae little difficulty was encountered by varying the salt concentration of the suspension, the organisms being fairly resistant to plasmoptysis. With the red pleomorphic rods much care to prevent rupture of the cells was required, the staining being correspondingly more difficult. The most generally satisfactory method was to add the culture to a large drop of 20% sodium chloride in a Petri dish, with little stirring. A loopful of this is transferred to a clean slide, gently spread and allowed to dry without heating. Immediately the smear is dry it is flooded with absolute methyl alcohol for two to three minutes, renewing if necessary. The excess alcohol is poured off and the stain added directly, without previous washing. For single stains 1% aqueous basic fuchsin was found to be most generally satisfactory, staining one to three minutes. Giemsa and aniline carbol gentian violet were also found useful. For flagella staining, Muir's modification of Pittfield's method was used. Instead of the absolute methyl alcohol used for the preliminary fixing, the acetic-alcohol fixative used by Browne (4) was occasionally found to be more suitable (one part glacial acetic and one part absolute alcohol).

For hide inoculation tests, fresh calfskin was used, clipped, cut into pieces 2 in. square, washed well with water and placed in Petri dishes. Sterile sodium chloride was added in excess. After inoculation water was added from time to time if necessary to prevent desiccation.

Isolation of Organisms from Hides

Three types of green salted hides showing discoloration were studied, Argentine steer hide, Canadian cowhide and Canadian buffalo hide.

Examination of discolored areas, dark pink in shade, on the Argentine hide, showed the organisms to be almost entirely micrococci, variously grouped in pairs, fours, or in irregular clusters with indication of sarcinal arrangement. Medium *A*, with 15 to 25% salt, proved the most suitable substrate for isolation, growth of reddish colonies becoming first apparent after four to five days' incubation at 37° C. with the higher salt concentration, and after two weeks on the 15% salt plates. Non-chromogenic, halophilic types also appeared in lesser numbers, though more abundantly with 15% than with 25% salt. Media containing lower concentrations of salt were unsuited to the isolation of the red organisms, permitting only a haphazard growth of contaminants which are suppressed by higher amounts of salt. Medium *B*, of the type frequently used for isolating organisms associated with reddening of salt fish, likewise permitted the growth of red halophiles, though less abundantly than Medium *A*. From both red and colorless colonies several strains were purified by replating three times on Medium *A* with 20% salt. Morphological and physiological comparisons of these purified strains indicated but one species of each type, a red sarcina (Culture 90-R5) and a non-chromogenic, non-sporing rod (Culture 90-N2).

The discoloration of Canadian cowhide was of a deeper reddish tint than that of Argentine hide, while the microscopic examination revealed a less uniform bacterial flora, which consisted mostly of rod-shaped organisms of various sizes with a small number of coccoid forms. That the organisms responsible for the reddening differed from those on the Argentine hide was further suggested by the cultural examination. Medium *A* (with 15 to 25% salt), which had been well adapted for the isolation of the red organisms in the latter, proved unsuitable for growing the red types on the cowhide, though it permitted good development of non-chromogenic organisms. On Medium *B* (with 20-25% salt) however, red growth occurred, appearing first after one week's incubation at 37° C. as small round pink colonies which gradually increased in size and deepened in color with age. It is of interest to note that on the fish medium (Medium *B*) little or no growth of non-chromogenic halophiles occurred so that the two media thus tend to be reciprocally selective for the two types of organisms. Microscopic examination of the red colonies showed rod forms with much pleomorphism, necessitating the use of a salt solution of approximately 20% for satisfactory observation. Mixed in water or weak salt solution, the organisms formed a viscous mass owing to distortion and rupture of the cells from plasmoptysis. Comparison of the isolated halophilic strains indicated one red type (Culture 91-R6) and two non-chromogenic rods (Cultures 91-N2 and 91-N4).

On buffalo hide the discoloration was slightly deeper in shade than that on the cowhide, though the microscopic picture was very similar, showing mostly rods of varied morphology with a smaller number of cocci. Media *A*

and *C* were used for isolation, the latter having been found superior to Medium *B* for culturing organisms of the pleomorphic rod type isolated from cowhide. As before, Medium *A* with 20% salt gave good growth of non-chromogenic halophiles together with smaller numbers of reddish colonies similar in type, though paler in color, to the sarcinae from the Argentine hide. On the milk salt agar (Medium *C*) with 24% salt, however, the beginning of reddish growth could be seen, after four to five days at 37° C., increasing in intensity of color until an abundant growth of deep red colonies was observed. As growth increased there was a marked zone of clearing round the colonies, pointing to a proteolytic action on the casein of the medium. Growth of non-chromogenic halophiles was almost entirely absent on Medium *C*, while colonies of the pink sarcinae developing on Medium *A* were not seen. Five halophilic types in all were isolated from the buffalo hide, of which two were reddish organisms, a sarcina (Culture 63-R1) and a pleomorphic rod (Culture 63-R2). Two non-chromogenic rod forms (Cultures 63-N1 and 63-N4) and one sarcina (Culture 63-N2) were also found.

Red Halophilic Organisms

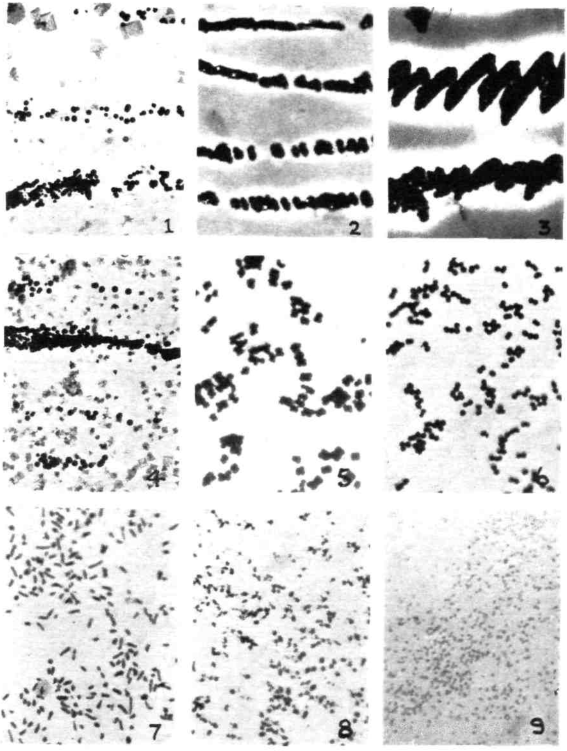
Culture 90-R5 (Sarcina litoralis Poulsen)

This organism, considered as the cause of red discoloration on the Argentine hide, is a large coccus. Cells may occur singly, in pairs, in fours, in short chains or in packets formed by division in three planes, the arrangement varying with such factors as medium, temperature, salt concentration and age of the culture (Plate I, Figs. 5 and 6). In young cultures (five days, 37° C.) on Medium *A* with 20% sodium chloride, cells vary from 1.2 to 1.6 μ in diameter with an average of 1.3 μ. There is a slight increase in size with higher salt concentration. The temperature of incubation has little effect on the size of the cells, though at temperatures of 20° or 28° C. there is more tendency to grow in packet formation than at 37° C. Less favorable salt concentrations likewise favor the sarcina form and larger packets. Medium *C* favors the growth of single cells, pairs, fours, and short chains more than the packet form (Plate I, Fig. 6).

The organism is non-motile, non-sporulating, and stains well with ordinary dyes. The reaction to the Gram stain is variable, with a rather larger proportion of positive than negative cells. It is an obligate halophile, growing at a salt range of 16 to 32% with an optimum of 20-24% sodium chloride. It is an obligate aerobe, with a temperature optimum of 37° C.

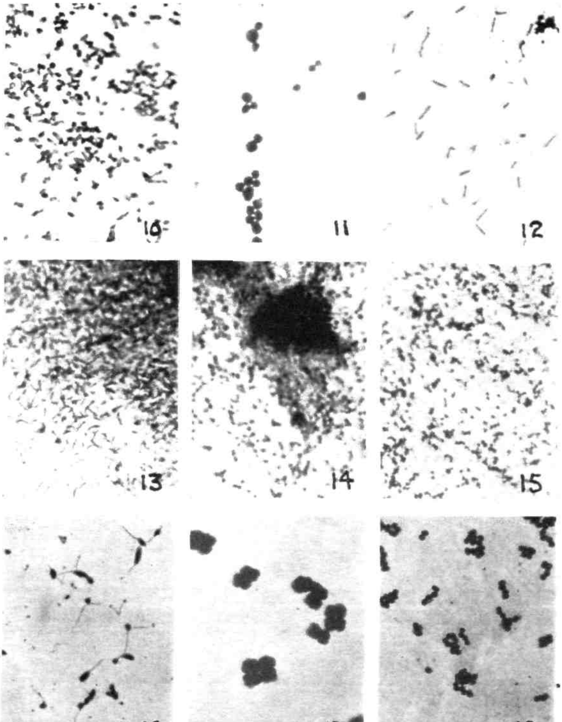
On Medium *A* with 20% salt at 37° C. the organism develops after three to four days. Nearly colorless at first the growth deepens in shade as it increases in size. Colonies are usually 1–3 mm. in diameter, round, entire, convex, with a waxy appearance, brick red with a pale border (Plate I, Fig. 1). Slant cultures are filiform, slightly raised, with entire edge and with a color indicated by Ridgway (16) as "coral red". There is a slight decrease in intensity of shade as the sodium chloride concentration and the temperature depart from the optima.

PLATE I



Magnification:—Figs. 1–4 natural size, others × 1000. Stain:—fuchsin, unless otherwise stated.

FIG. 1. *Sarcina litoralis. Colonies, beef-yeast extract agar, 20% salt, 4 weeks.* FIG. 2. *Serratia salinaria. Milk agar plate culture, 24% salt, 4 weeks, showing proteolysis.* FIG. 3. *Serratia cutirubra. Milk agar plate culture, 24% salt, 4 weeks, showing marked proteolysis.* FIG. 4. *Sarcina sp.* (*Cult. 63-R1.*) *Colonies, beef-yeast extract agar, 20% salt, 4 weeks.* FIG. 5. *Sarcina litoralis. Beef-yeast extract agar, 20% salt, 15 days, 28°. Packet form.* FIG. 6. *S. litoralis. Milk agar, 20% salt, 13 days. Staphylococcal form.* FIG. 7. *Serratia salinaria. Milk agar, 24% salt, 5 days, 37°. Rods in young culture.* FIG. 8. *Ser. salinaria. Milk agar, 24% salt, 14 weeks. Coccoid forms, old culture.* FIG. 9. *Ser. salinaria. Milk agar, 20% salt, 10 days. Small cocci, dwarfed growth* (*Giemsa stain*).



Magnification, × 1000. Stain:—fuchsin, unless otherwise stated.

FIG. 10. *Ser. salinaria. Milk agar, 28% salt, 10 days. Rod form, staining irregularly.* FIG. 11. *Ser. salinaria. Starch agar, 20% salt, 3 weeks. Large coccoid form (Gonidangia) with budding.* FIG. 12. *Serratia cutirubra. Milk agar, 24% salt, 5 days, 37°. Rods.* FIG. 13. *Ser. cutirubra. Milk agar, 32% salt, 10 days. Rod forms disintegrating and coalescing to form symplastic mass.* FIG. 14. *Ser. cutirubra. Milk agar, 28% salt, 10 days. Granular symplasm and coccoid form.* FIG. 15. *Ser. cutirubra. From inoculated hide, red area, 12 weeks, 20°. Small cocci.* FIG. 16. *Ser. cutirubra. Milk agar, 28% salt, 6 days, 37°. Flagella. (Muir-Pittfield stain).* FIG. 17. *Sarcina sp. (Cult. 63-R1.) Beef yeast-extract agar, 20% salt, 11 days, 20°. Packet form.* FIG. 18. *Sarcina sp. (Cult. 63-R1.) Milk agar, 20% salt, 18 days. Staphylococcal form.*

No growth is obtained on ordinary media. The organism grows, though less readily, on standard nutrient agar with 20% salt and on milk salt agar. On the latter there is no evidence of proteolysis. On salt gelatin growth is definite though slow, with no liquefaction. Diastatic action is negative. In lead acetate agar growth is very slight, though a slight blackening is evident near the surface. Nitrates are reduced to nitrites. On potato (in 20% sodium chloride) growth is very scanty, appearing as a slight chalky, pink development near the top. Liquid media are unsuited to the organism, growth being negative or extremely scanty even with suitable salt concentration. On salted calfskin it causes reddening after one week at 37° C.

This organism appears to be closely related to other sarcinae isolated from various salted products. Poulsen (15) first described the isolation of a large red, salt-loving coccus which he named *Sarcina litoralis*, with which *Sarcina morrhuae* Farlow (6), isolated from reddened codfish, is apparently identical. The organism studied by Kellerman (9) and designated by him *Micrococcus litoralis* (syn. *Sarcina litoralis* Poulsen) is likewise similar to the type here reported, though he found it appearing chiefly as diplococci and single cocci. As the arrangement of sarcinae may vary with the cultural conditions, however, this feature is not considered sufficient to warrant a change of generic name. Klebahn (10) reports the isolation of a large halophilic coccus from reddened codfish which he classified as *Sarcina morrhuae*, while more recently Liebert and Deerns (11) and Petter (14) also describe types likewise isolated from fish which they consider similar. Another closely related species is the halophilic "red coccus" isolated by Cloake (5) from salted fish with red discoloration, and which was found to occur in both staphylococcal and sarcinal form. Our sarcina appears, moreover, to be closely related to the red sarcinal strains isolated by Lloyd and coworkers (12, 17) from salted hides showing "red heat" and from marine curing salts of different origin. These were also strongly aerobic halophiles, growing best at 37° C. and nonliquefying.

It is apparent that red halophilic sarcinae, similar in type to the writer's organism from Argentine hide, have been isolated from various salted products. As far as a comparison of characteristics permits the writer is inclined to classify the type as *Sarcina litoralis* Poulsen, a name which has priority over the designation *Sarcina morrhuae* employed by various authors for evidently similar organisms.

Culture 91-R6. Serratia salinaria (Harrison and Kennedy) Bergey et al.

This organism, regarded as the active agent in the discoloration of salted cowhides examined, is highly pleomorphic (Plates I and II, Figs. 7-11). It may occur as long rods and as small or large cocci with a wide range of intermediate forms which may be oval or of irregular shape and of varying size. Young cultures on milk salt agar with 24 to 28% salt show abundant rod forms of varying length which grade into coccoid cells, the rods ranging from 1 to 6 μ in length and from 0.6 to 1.5 μ in width, and the diameter of the round cells varying from 0.8 to 1.4 μ (Plate I, Fig. 7). Rod forms are

relatively scarcer in older cultures; here round cells, more or less irregular in shape and size, predominate (Plate I, Fig. 8). The form and size of the cells are influenced by the nature of the basic medium, salt concentration and temperature. An increase in salt concentration results in a slight increase in cell size and tends to favor the elongated form. Increasing the temperature up to the optimum (37° C.) produces similar effects. In addition to the smaller vegetative cocci, larger round forms may occur, 4 to 7 μ in diameter, with a pronounced granular appearance, and which may break up into a granular symplasm. This stage may be seen readily where the organism is cultivated on a medium of coagulated egg albumin with salt and yeast extract.

Apart from the methods of fission and also, apparently of upgrowth of regenerative units, the organism appears to be capable of reproducing by budding. This is observed on three-week cultures on starch agar with 20% salt, on which the organism grows slowly. Cultivated at 37° C., the organism forms large, round cells, apparently functioning as gonidangia, varying in diameter from 2 to 7 μ (Plate II, Fig. 11).

All stages of the organism are gram-negative. In the rod form it is motile while in young cultures oval cells and even round forms may be seen to be provided with flagella, indicating a stage in the life cycle analogous to that of the "swarmers" noted, *e.g.*, in *Rhizobium*. The oval or rod forms possess a single polar flagellum or frequently two flagella, one at each end, the motile coccoid forms showing a single organ. Flagella may reach a length of 10 μ. The large coccoid forms referred to above do not appear to be motile.

No growth of the organism occurs on ordinary media, and little or none even with the addition of salt. Special media are required, such as milk-salt agar, coagulated albumin (with yeast and salt), and fish-salt agar. Definite, though slow growth occurs on salt gelatin with beef and yeast extract. On milk-salt agar with 24% or more of salt, growth develops after three to four days at 37° C., pinkish at first but soon showing a definite red color corresponding to "scarlet" on Ridgway's chart. Colonies usually reach a diameter of 4 to 5 mm. Slant cultures are filiform, but slightly raised, with smooth, glistening surface and of butyrous consistency. As the culture ages a definite zone of proteolysis becomes apparent surrounding the growth (Plate I, Fig. 2). Cultures at this stage show an increase in pH value and a strong reaction for ammonia. There is also a characteristic putrefactive odor from such cultures, similar to that produced on calfhide by inoculation, in which case reddening occurs within four days at 28 to 37° C.

The organism develops at a range of 20 to 32% salt, with optimum growth between 28 and 32%. The temperature optimum is 37° C. The organism is an obligate aerobe. At 20° C. liquefaction of gelatin is slow but definite. Little or no growth occurs in liquid media. It does not reduce nitrates, nor does it exhibit any diastatic action. In stab cultures of lead-acetate-salt agar there is but very scant growth with a trace of blackening near the top. No growth has been observed on potato.

A comparison of the morphological and cultural characteristics of the organism with those of reported red halophiles suggests identity with *Serratia salinaria* (8) found on reddened codfish. This latter organism showed not only similar pleomorphic forms, but exhibited closely similar physiological properties to such a degree that the two are regarded as specifically the same.

Culture 63-R2 Serratia cutirubra n.sp.

This organism, the causal agent of red discoloration on buffalo hides, is likewise pleomorphic (Plate II, Figs. 12-16). Young cultures on milk-salt agar (five days, 37° C.) showing forms varying from round cells, 1 to 1.5 μ in diameter, to rods 1.5 to 8 μ long by 0.7 to 1.4 μ wide (Plate II, Fig. 12). Rods predominate in young cultures, with older cultures showing an increasing proportion of coccoid forms. Apart from the question of age, the rod form is favored by higher salt concentration and by cultivation at 37° C. In the rod form the cells have a greater average length than *S. salinaria*. One-month cultures show almost exclusively coccoid cells, 0.8 to 1.5 μ in diameter, the appearance being very similar to that of the organism inoculated on to calfhide and incubated several weeks (Plate II, Fig. 15). Under certain conditions (*e.g.*, in milk agar with 32% salt) the organism, through disintegration of cells, forms a granular symplasm from which the granules or "regenerative units", by transference to a favorable environment, may form vegetative cells. As with *S. salinaria*, there is indication of a further mode of reproduction by budding of large coccoid cells, 1 to 2 μ in diameter, noticeable on media on which growth is comparatively slight (*e.g.*, starch-salt agar). In the rod form the organism is motile by means of a single polar flagellum (Plate II, Fig. 16). Motility may be observed in coccoid cells in young, though not in old, cultures. The organism stains but moderately well with ordinary dyes, and is consistently gram-negative.

No growth occurs on ordinary media, nor on most ordinary media with salt. Special substrates are required for good development, the best of those tried being Medium *C*. On this it grows at a salt concentration range of 20% to saturation, developing best with 28 to 32%. At 37° C., growth appears after three to four days, showing first pinkish in color, but developing into a deeper color corresponding best to "rose dorée" of Ridgway. Single colonies may reach a diameter of 3–4 mm., round and slightly convex. Slant cultures show filiform growth, slightly spreading, rather flat with smooth, glistening surface and membraneous consistency. Apart from differences in color and consistency the organism is further distinguished from *S. salinaria* by its stronger proteolytic action on milk, giving a much more rapid and extensive zone of clearing (Plate I, Fig. 3). The odor is pronounced, similar to that when inoculated on hide, on which typical reddening begins after 4–5 days.

The organism grows moderately on salt-fish agar, coagulated albumin (with yeast and salt) and slightly on starch yeast-salt agar and salt gelatin. Liquefaction of gelatin is pronounced. Nitrates are not reduced, diastatic action is negative while no indication of carbohydrate fermentation is seen. Indol is not formed. Little or no growth occurs in liquid media. The organism is an obligate aerobe, with a temperature optimum of 37° C.

While the organism is of the same group as *S. salinaria* it shows consistent differences in morphology and cultural character, particularly as regards color and consistency. These features, in addition to its more active proteolytic properties, appear to justify separate classification, and consequently the name *Serratia cutirubra* n.sp. is suggested for the species.

Technical description—Serratia cutirubra n.sp.

Pleomorphic rod, occurring also as oval and coccoid forms and in symplastic stage; rods 1.5 to 8 μ long by 0.7 to 1.4 μ wide; cocci 1 to 2 μ in diameter; in young cultures motile with one polar flagellum; no spores; Gram-negative; obligate aerobe; obligate halophile growing in 20% sodium chloride to saturation, best from 28–32%; no growth on ordinary media; little or none in liquid media with salt; grows best on milk-salt agar or fish-salt agar with colonies round, reddish (rose dorée), convex, glistening, and membraneous; optimum temperature 37° C.; liquefies gelatin; digests casein; produces ammonia, and hydrogen sulphide; nitrates not reduced; indol not formed; diastase negative; no fermentation of carbohydrates; produces reddening on salted calfhide.

Culture 63-R1 Sarcina sp.

In addition to the organism just described, a reddish sarcinal type was isolated from discolored buffalo hide (Plate I, Fig. 4; Plate II, Figs. 17 and 18). Morphologically and physiologically it resembled *Sarcina litoralis*, the active agent on Argentine hide. It differed from the latter in showing a paler shade of red in cultures, and in developing at a slightly lower maximum salt concentration, namely, 28%. Inoculation tests on salted calfhide did not produce reddening, and it is therefore not regarded as an active agent in causing this defect under practical conditions.

Non-chromogenic Halophiles

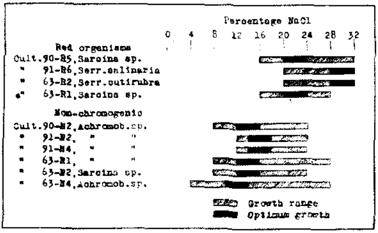


FIG 1. *Relation of salt concentration to growth of halophilic organisms from hides. (Percentage sodium chloride = gm. per 100 c.c. medium).*

In addition to the red halophiles, a number of non-chromogenic types were likewise isolated from the various discolored hides. Since this paper is concerned primarily with organisms producing red discoloration, a detailed description of their characteristics is omitted. The chief morphological and cultural features, however, are summarized in Table I (see also Fig. 1).

Observations

The question of the actual damage to hides caused by red discoloration appears to be a controversial one. It is probable that the injury depends upon the type of organisms concerned and particularly upon their proteolytic activity. Becker (2) regarded red discoloration as harmless since the organisms studied were non-liquefying. Furthermore Lloyd and coworkers found their red organisms to be likewise non-liquefying and less liable to damage the hide than proteolytic types. On the other hand, the signs of damage reported in Vestnik (1) were said to be attributable to proteolytic bacteria, while Stather and Liebscher (20) found indication of definite injury.

The writer's observations support the view that hide injury in red discoloration is dependent on the proteolytic action of the bacteria. Thus in the case of the Argentine hide examined, the red organism, similar in type to those studied by Lloyd *et al.* (12, 17), was non-liquefying and did not show signs of proteolysis on hide. On the other hand, the two species of *Serratia* isolated from Canadian hides and both liquefying types, though in different degree, are considered capable of greater damage, inoculation on fresh salted hide resulting in definite proteolytic action. The non-chromogenic types isolated, some of which are liquefying, develop at a lower salt concentration range than the red organisms, and probably show little activity in a medium approaching saturation. While associative action with the red bacteria is possible, it was not indicated by inoculation tests.

TABLE I

CHARACTERISTICS OF NON-CHROMOGENIC HALOPHILIC ORGANISMS

Culture no.	Form	Size, μ (Med. *A*, 20% salt)	Motility	Spores	Gram stain	Opt. temp.	Growth on Med. *A* (with salt)	Growth on Med. C (with salt)	Salt conc. range, % (*Med. A*)	Opt. salt conc., %	Growth on potato (in brine)	Gelatin liq.	Nitrate red
90-N2	rod	0.6–5.0 ×0.4–0.5	–	–	±	37	+	–	8–24	12–16	–	+	–
91-N2	rod	1.0–4.0 ×0.4	+	–	–	37	+	–	12–24	16	–	+	–
91-N4	rod	0.3–3.5 ×0.5	+	–	±	37	+	–	12–24	16	–	+	–
63-N1	rod	1.2–3.5 ×0.4–0.5	+	–	–	37	+	sl.	8–28	12–16	+	–	+
63-N2	sarcina	1.0–1.2	–	–	±	30	+	–	8–24	12–16	–	–	–
63-N4	rod	1.4–4.0 ×0.6–0.7	+	–	–	28	+	sl.	4–28	12	–	–	–

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TRICHOGRAMMA MINUTUM RILEY AS A PARASITE OF THE ORIENTAL FRUIT MOTH (*LASPEYRESIA MOLESTA* BUSCK.) IN ONTARIO[1]

By W. E. van Steenburgh[2]

Abstract

The investigations described in this paper were carried out during the period 1928-1933, and deal with the egg parasite, *Trichogramma minutum* Riley, with special reference to its field of usefulness in the biological control of the oriental fruit moth (*Laspeyresia molesta* Busck.) in Ontario. This cosmopolitan parasite is a factor of considerable importance in regulating the abundance of the fruit moth in southern New Jersey, but appears to be of little practical significance in the natural control of the pest in Ontario. Under certain conditions of weather and host abundance, parasitism may be increased materially by the liberation of *Trichogramma* in the orchards but, in general, the results are not dependable. A large number of experiments were conducted utilizing three biological races of the species. The technique employed in the work and the results obtained are given as well as a number of important observations on the habits and biology of the parasite.

Introduction

The studies with *Trichogramma minutum* Riley, described in the following discussion, were undertaken to determine the usefulness of this small egg parasite as a factor in the biological control of the oriental fruit moth (*Laspeyresia molesta* Busck.) in Ontario. This serious pest was accidentally introduced into the Niagara Peninsula about 1922, and three years later was causing considerable damage to the peach crop in the vicinity of St. Davids, Ont. By 1927 the moth had spread over a large area and presented a serious problem to the peach growers of the district.

Trichogramma, a native insect, doubtless attacked eggs of the oriental fruit moth prior to 1928, when these studies were initiated, but its occurrence, so far as this host is concerned, had not been recorded, and little was known about the habits of the parasite, its actual distribution, or its importance as a control. The natural studies and liberations of the laboratory-bred material were undertaken to determine the value of the species under natural conditions and as a possible artificial control.

The original laboratory breeding stock of *Trichogramma* was obtained from Louisiana and California and belonged to Flanders' so-called "gray race". The field releases of 1928 and 1929 were made entirely with this strain. In 1930 and 1931, the native (yellow) race was reared in the laboratory and used in orchard releases. In 1931, an additional native strain, much darker in color (not listed by Flanders but studied by Martin (8) in Michigan), containing a varying percentage of wingless males, and found attacking the eggs of *Sialis infumata* Newm. in eastern Ontario, was used in the work. In 1932

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and 1933, the orchard releases were confined to the yellow indigenous race. Each race appeared biologically distinct and attempts to cross fertilize them failed. The laboratory and field studies showed considerable variation in activity and responses and indicated the possibility of each strain being suited to a particular environment. The developmental period of the races varied somewhat. At 80° F. the native yellow strain required eight days from egg to adult; the gray strain eight days and six hours; and the native dark strain slightly over nine days. In each case the parasitized host eggs became pigmented to a dull black during the third day.

The oriental fruit moth has three generations in Ontario, and in some years a partial fourth. The eggs of the first generation appear during the last two weeks of May and throughout June, with the maximum oviposition generally appearing in early June. The eggs of the second generation are deposited in July with the majority appearing during the latter half of the month. The maximum oviposition of the third generation appears in late August or early September and the hatching larvae enter the maturing fruit. It is the larvae of this generation which seriously damage the fruit. Eggs appearing at a later date in the season are of no consequence in fruit damage and are of no concern in this study except possibly as winter hosts for the parasite.

During most seasons, the indigenous *Trichogramma* do not attack eggs of the oriental fruit moth on peach until after the peak of the second generation of eggs and, therefore, have no opportunity materially to increase their numbers until the eggs of the third generation are on the foliage. The season is then too far advanced for an effective parasite population to develop. This natural handicap may possibly be overcome by the early colonization of laboratory reared parasites.

The efforts to secure control by the release of laboratory reared *Trichogramma* described in this paper are of an experimental nature. Suitable methods of colonization, the time of season best suited to the releases, and the number and distribution of the parasites within the orchards have been some of the more important subjects of study.

The Natural Appearance of *Trichogramma*

To secure a proper background for the experiments on colonization of laboratory reared *Trichogramma*, a knowledge of the seasonal appearance and normal abundance of the parasite in oriental fruit moth eggs was needed. This information was obtained by making weekly examinations of moth eggs at definite points through the infested area.

From 1928 to 1930 native *Trichogramma* was unimportant in reducing the numbers of eggs of the oriental fruit moth. This was chiefly due to its late appearance in the orchards, the small numbers of the first arrivals, and insufficient time to build up an effective parasite population. In these years only two or three generations of the parasite lived in this host before the fruit was picked. In 1931, field conditions were different. Native *Trichogramma* began its attack on eggs of the oriental fruit moth in early July and soon became abundant and remained an important factor of control through-

out the season. In 1932 and 1933 natural *Trichogramma* made an early appearance in the orchards, but with one exception was of little importance in reducing the injury caused by the pest.

Only two fruits in Niagara, the peach and quince, were consistently chosen for moth oviposition. During the earlier part of the season moth eggs were more abundant on quince, generally near the developing fruit where often as many as 30 or 40 eggs were found. With the exception of 1931 and 1932, there was a gradual decrease in preference for quince as the season advanced, with a corresponding increase in oviposition on peach. In 1931 and 1932, eggs were fairly plentiful on quince throughout the season and the usual increase on peach did not develop. In each year *Trichogramma* was abundant where host eggs were most plentiful.

Figs. 1 to 4* show the general trend of oviposition of the oriental fruit moth for the years 1928 to 1931, and, therefore, indicate the abundance of this host available for parasitism in the various egg generations. The information for the 1928 and 1931 graphs was taken from the St. Davids' records, while the 1929 and 1930 graphs were compiled from data collected at Virgil and Niagara-on-the-Lake. In general, the conditions at these points are very similar. The 1929 and 1930 records were taken from Virgil and Niagara-on-the-Lake so that no influence would be felt from the artificial colonization experiments at St. Davids.

A period, equivalent to part of a parasite generation before the first actual field record was taken, was allowed on the charts, since, in most cases, the parasite was in the later stages of development when first recorded. The curve for percentage of parasitism is drawn in proportion to the total number of host eggs present.

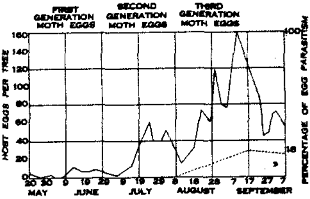

FIG. 1. *Oviposition of oriental fruit moth on peach and the natural parasitism by Trichogramma minutum Riley, 1928 (—— host, ... parasite).*

In 1928, *Trichogramma* was first taken parasitizing eggs of the oriental fruit moth on quince during the latter half of July. On July 23, 13% parasitism was found at Niagara-on-the-Lake. By August 10, a similar count at the same point showed 21%. The peach orchard at St. Davids failed to show the presence of the parasite on August 3, but a late count, made after the peaches were picked, showed 15%. Since, as will be shown later in the paper, the egg shells from which parasites emerge fall from the foliage sooner than do normally hatched eggs, it appears that the actual parasitism on the third generation of eggs would be at least 18 to 20%.

* *The egg graphs are a compilation of bait-pail moth catch and insectary oviposition records furnished by Mr. W. A. Ross and staff, Entomological Laboratory, Vineland Station, Ont., and egg counts secured from the foliage. The tree oviposition records and percentages of parasitism for 1928 were furnished by Mr. C. W. Smith.*

The first appearance of *Trichogramma* in eggs of the oriental fruit moth during 1929 was from quince at Niagara-on-the-Lake. Here, on July 29, the parasitism was 11·3%. A count made on peach foliage 100 yards away failed to show any parasitized eggs. *Trichogramma* was not found elsewhere in the district at this time. By August 14 the parasitism on quince foliage at Niagara-on-the-Lake had increased to 22%, while records from Queenston and Stamford showed respectively 13 and 18%. On August 15 a parasitism of 18% was found on peach foliage at Stamford, just across the highway from a quince planting. The following day a record from peach at Virgil showed 14% egg parasitism. By September 4 egg parasitism had increased to 20% at Virgil and was 16% at Niagara-on-the-Lake. The final counts made on eggs of the third generation, just previous to the picking of the late varieties of peaches, gave 40 to 45% parasitism at Virgil and 16% at Niagara-on-the-Lake. It should be noted that eggs were very plentiful in one of the Virgil orchards.

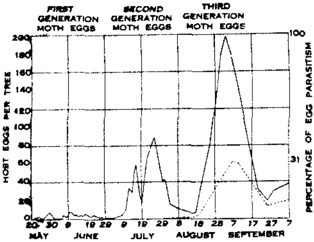

FIG. 2. *Oviposition of oriental fruit moth on peach and the natural parasitism by Trichogramma minutum Riley, 1929 (—— host, ... parasite).*

The field conditions during the first generation of the oriental fruit moth in 1930 pointed to the possibility of a heavy increase of eggs in the following generations. Dry weather, and the sudden appearance of large numbers of chrysopid larvae on the peach and quince foliage, seriously affected its seasonal history. Instead of a sharp increase in egg abundance in the third generation, there were few more than during the second generation. This scarcity of host material and the presence of the predacious chrysopid larvae had a very retarding effect on the increase of the egg parasite so that, in many orchards, they were found only with difficulty.

The first seasonal record of *Trichogramma* was on quince at Niagara-on-the-Lake on July 16, when an egg parasitism of 5% was observed. Counts at other points did not show the presence of *Trichogramma*. By July 30 the parasitism on quince at Niagara-on-the-Lake had increased to 39%, which represents the highest record for parasitism for the year. Thereafter, it diminished on quince until at the same point on September 1 no parasitized eggs could be found. The first record on

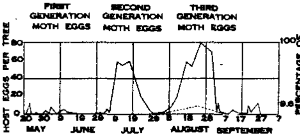

FIG. 3. *Oviposition of oriental fruit moth on peach and the natural parasitism by Trichogramma minutum Riley, 1930 (—— host, ... parasite).*

peach was on August 18, when a parasitism of 3.4% was obtained at Virgil; this increased to 13.3% just before the time of picking. *Trichogramma* was not secured from other points until the peak of eggs of the third generation, when 6% was found at Niagara-on-the-Lake.

In 1931, *Trichogramma* was seen parasitizing eggs on quince at Niagara-on-the-Lake on June 24, when 15% egg destruction was found. At this place egg parasitism had increased on quince to 42.5% by July 15. Thereafter, throughout the season, egg parasitism remained slightly over 30% on this fruit. The first record for peach was on July 9 at St. Davids, when 22.4% was recorded. By July 22 parasitism had reached 33%. This was the highest record secured until September 3, when egg parasitism reached 36.5%. *Trichogramma* was abundant everywhere throughout the peach growing district and was an important factor in moth egg destruction.

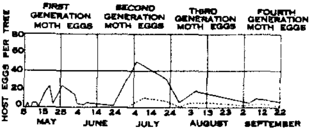

FIG. 4. *Oviposition of oriental fruit moth on peach and the natural parasitism by Trichogramma minutum Riley, 1931 (—— host, ... parasite).*

After 1931, the decreased number of eggs of the oriental fruit moth present in the orchards and the increased time spent in the colonization orchards made continued weekly observations difficult. Occasional observations gave the following information for 1932. Egg parasitism to the extent of 10% was found on the first eggs appearing on quince on June 1. It increased on quince until an egg parasitism of 38.3% was reached during the last week of June, after which it progressively diminished throughout the remainder of the season. On June 14 an egg parasitism of 7.7 and 15.9% was found in two peach orchards at St. Davids. In one of these orchards a collection of second generation eggs showed 27.2%, which was exceptionally high, as compared with the general average for the district of less than 5% on this generation of eggs. One heavily infested orchard near Hamilton showed a natural egg parasitism of 63.5% on third generation eggs, which was the highest record secured for natural *Trichogramma* throughout the study. In general, third generation egg parasitism this year varied between 0.0 and 23.0%.

In 1932, an experiment was designed to show the distribution of *Trichogramma* within an orchard in the early part of the season. A large orchard, adjacent to woodland, was chosen and egg examinations made at regular distances from the outside of the orchard inward. Two trees on the edge of the orchard gave 24.2 and 53.2% egg parasitism. Seventy-five yards from the edge of the orchard the parasitism had dropped to 12.2%, while the main body of the orchard, over 100 yards from the edge, gave 7.7%. A definite dispersal trend was apparent from the woodland into the orchard. This trend was also verified by the use of artificially placed *Sitotroga* eggs.

The first records of natural *Trichogramma* for 1933 were obtained on May 28 in peach orchards in the studies conducted with artificially placed *Sitotroga* eggs at St. Davids and Niagara-on-the-Lake. The highest percentage of egg parasitism observed was five, but even this low concentration would be sufficient to build up a parasite population which would be of importance in control. Such was not the case and later examinations in the same orchards toward the end of the first generation of host eggs failed to show any increase, and, throughout the remainder of the season *Trichogramma* continued to be of little importance. In this connection it should be noted that the moth infestation in this district was lower than in any year since the studies began. No orchard examined throughout the remainder of the season showed a higher egg parasitism than 15.7%, with the exception of the orchard mentioned for 1932 near Hamilton, and here a 28.8% parasitism was found on third generation eggs.

Trichogramma parasitism on quince was 24.7% at Niagara-on-the-Lake on June 13 and by July 9 the parasitism had increased to 31.4%. Throughout the remainder of the season a decreased activity was observed on this fruit.

In 1933, natural *Trichogramma* was of little value as a control agency. This decreased activity may, in part, be explained by the general decrease in the available eggs of the oriental fruit moth.

The activity of the native *Trichogramma* on eggs of the oriental fruit moth may be summarized in the following remarks:

(i) Only the native yellow race of *Trichogramma* appears naturally attacking this host.

(ii) *Trichogramma* is very irregular, both in its seasonal appearance and sectional distribution. It may be abundant in certain sections within a district and almost entirely absent in others. This was particularly noticeable in 1929, when the parasite was generally abundant, but showed a low concentration near Queenston and St. Davids. It is suggested that the abundance and distribution of native spring hosts regulates its later appearance in the orchards.

(iii) The percentage of egg parasitism varies considerably in different orchards and from year to year. This variation seems dependent upon two factors, (1) the degree of host infestation as expressed in egg abundance, and (2) the number of *Trichogramma* migrating into the orchards from native hosts. The latter factor has remained quite constant, with the exception of 1931, and, in general, the percentage of eggs destroyed has varied directly with the number of host eggs available. This is partly explained by the habits of the parasite. In the presence of an abundance of host eggs the movements of the female parasites are localized, but when eggs are scarce, most of the parasite's effectiveness is dissipated in rapid movements and migrations.

(iv). Except in years such as 1931, little assistance in the form of a high percentage of destruction of eggs of the oriental fruit moth is to be expected from the indigenous *Trichogramma*. It may play an important role in the more heavily infested orchards, but, on the whole, a high percentage of egg parasitism is not likely.

Overwintering

The following studies were conducted during the fall and winter months of 1928 and 1929, and 1929 and 1930 to determine the possibility of *Trichogramma* overwintering in eggs of the oriental fruit moth oviposited in late fall. The availability of this host offered the possibility of having a ready supply of *Trichogramma* for early emergence in the orchards.

The first series of studies consisted of fallen leaf collections made late in the fall of 1928 from different orchard situations as follows: (i) beneath the trees; (ii) from the sides and bottom of the orchard drainage ditches; (iii) from among the tall grass along the edges of the orchard. The leaves were stored in an open insectary until such time as they could be examined during the winter. From several thousands of leaves, only five unhatched parasitized eggs were found and these failed to emerge.

In the following year parasitized eggs of the oriental fruit moth of varying ages, reared at different temperatures, were exposed to the late fall and early winter climate. These had all perished by the first week of January. An examination of the dead parasites suggested death through desiccation.

Eggs of the oriental fruit moth do not appear to be a suitable overwintering host for *Trichogramma* in Ontario. The egg chorion is too fragile to protect the contents of the egg during such long periods of sub-development weather.

The Seasonal History of *Trichogramma*

This phase of the work was to provide a means of determining the number of life cycles of *Trichogramma* during the season, the possible number of generations following a colonization of material reared in the laboratory, and also the factors influencing the developmental rate. In 1929, successive generations of the gray race were reared on peach foliage, and in 1930 the work was repeated with the native yellow race. From a comparison of the results of these studies with the developmental curve of the parasite obtained from laboratory rearings at constant temperatures, it was shown that temperature was the only important weather factor influencing the rate of development. By comparing the mean temperatures for a particular period with the developmental curve, as obtained from the field life cycles and laboratory rearings, the number of possible generations of *Trichogramma* could readily be determined.

The time required for a cycle in the orchards at any given average temperature compared quite favorably with the laboratory rearings at constant temperatures, but were, in general, slightly longer in the orchards. This may have been due to the more exposed position of the instrument shelter, and the fact that the development of the parasite on the foliage may have been slightly retarded by the cooling effects of the normal leaf transpiration.

The field cycles were conducted in the following manner. A 20-mesh screened cylindrical cage, some 10 in. in diameter and 14 in. long, was used. This cage had an unbroken cloth covering over one end and a cloth sleeve fastener fitted to the other end. A suitable branch was chosen on a level

TABLE I
GENERATIONS REARED USING THE GRAY STRAIN, 1929

No.	Started	Emerged	Length in days	Mean temperature, °F.
1	May 16	June 17	32	59.3*
2	June 17	July 1	14	72.0
3	July 2	July 16	14	72.6
4	July 17	July 30	13	73.7
5	July 31	Aug. 18	18	67.2
6	Aug. 18	Sept. 2	15	70.7
7	Sept. 2	—	—	— **

* *No. 1 was run with Ephestia eggs in the weather instrument shelter. Before May 16, there were only seven days with a mean temperature of over 50° F., which would allow little parasite development, since this temperature is close to the threshold of development for the species.*

** *This cycle had not emerged by September 15, when most of the peaches were picked.*

with the weather recording instruments and the screened cage was placed over the foliage. Female moths were introduced into the cage. When a suitable number of eggs was secured, the cage was removed and the *Trichogramma* adults liberated among the leaves. The eggs were then kept under observation until the next generation of parasites appeared. In obtaining the average mean temperatures for the developmental period, the daily mean for both the liberation day and the emergence day was included in the total average.

Table I shows the generations reared in 1929, using the gray strain.

It is seen from the rearings that there was a possible total of seven parasite cycles with six complete generations before the peaches were removed from the trees.

In 1930, the native strain was used for the life cycles. The first generation of the parasite was reared on *Ephestia* eggs in the weather instrument shelter, located in the orchard. Thereafter, the cycles were conducted on peach foliage. The generations are shown in Table II.

It will be seen that possibly there were eight effective generations of the parasite before the fruit was picked.

TABLE II
GENERATIONS REARED USING THE NATIVE YELLOW STRAIN, 1930

No.	Started	Emerged	Length in days	Mean temperature, °F.
1	May 1	June 3	33	59.5*
2	June 3	June 21	18	67.0
3	June 22	July 8	16	68.8
4	July 8	July 21	13	72.4
5	July 21	Aug. 1	11	75.2
6	Aug. 1	Aug. 13	12	74.2
7	Aug. 13	Aug. 30	17	68.5
8	Aug. 20	Sept. 17	18	67.6

* *There were three isolated days before May 1 with a mean temperature above 50° F.*

No connection could be observed between the mean relative humidity and the development of *Trichogramma*, except in periods of slow development accompanied by low atmospheric moisture, and under such conditions that the host egg became desiccated and the enclosed parasite was killed.

The period of effective temperatures for *Trichogramma* in Ontario may be five months. Actually on the peach foliage, there are three and a half months during which *Trichogramma* may develop before the peaches are picked and during this period six to seven generations are possible.

The Factors Affecting the Development and Behavior of *Trichogramma*

The studies included under this general heading were designed in an effort to evaluate the various races of *Trichogramma* in regard to control of oriental fruit moth in Ontario, and also to assist in interpreting the activity of the parasite in the field. The investigations are a combination of laboratory and field studies. The laboratory work was conducted in specially constructed constant temperature incubators, and the field observations were made in the peach orchards.

The general habits of the native dark race made it appear unfit for orchard liberations, and the following studies were conducted entirely with the imported gray and the native yellow races.

Eggs of *Ephestia kuehniella* Zell. were used in the laboratory experiments.

The Developmental Period at Different Constant Temperatures

The laboratory observations on the developmental period at different constant temperatures were made for comparison with the mean developmental temperatures obtained in the field studies. The temperature stations were chosen at five-degree intervals from 50 to 100° F. Below 55 and above 95° F. no development was obtained. In the former case the eggs became desiccated and, therefore, 55° F. does not necessarily represent the temperature threshold of development. The results of these studies are shown in Fig. 5. For comparative purposes, the developmental curve of the yellow strain of southern New Jersey, as determined by Peterson (9, 10), is included. The same general developmental trend is shown, with the New Jersey strain requiring less time to reach the adult stage at the various temperatures.

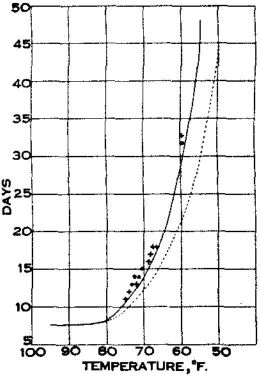

FIG. 5. *Developmental curve for Trichogramma minutum Riley. The solid line represents the developmental curve for the native yellow strain. The broken line represents a similar curve for the yellow strain of New Jersey for 1928 (Peterson). The points represent the gray strain reared in the orchard in 1929, and the crosses the yellow strain reared in the orchard in 1930.*

The gray race showed a slightly longer developmental period than the native yellow strain. This difference varied from several hours at the higher temperatures to slightly over a day in the lower temperature range. A close correlation was shown between the laboratory and field life cycles, which further substantiates the previous statement that temperature is the important factor determining rate of growth.

The females of the indigenous yellow race maintain their yellow coloration at *all* temperatures above 70° F. Below this, they show gray markings which become darker as the rearing temperature is lowered. The males of this strain show gray markings at all temperatures. The gray race retained its gray markings at all temperatures in both sexes.

Individual Female Ovipositions

Recently emerged females were isolated on embryological watch glasses 1½ by 4 cm. with ground glass tops which were held securely in place by elastic bands. The host eggs were pasted to a small round card which fitted part way down the concave sides of the dish. It was found convenient to face the eggs on the card downward and place a raisin between the upper surface of the card and the glass cover. The raisin provided food and moisture and was an important factor in offering a suitable environment. Parasites in dishes without such food soon perished, making much fewer ovipositions. The eggs were changed daily and new raisins were added as they became dry. A constant light was used over the incubators throughout the experiments, and conditions, except for the temperature, were kept constant. The generations of the two strains (gray and yellow) were reared side by side under identical conditions. The stock supplying the females for the work was reared at 80° F. and only mated females were used.

TABLE III

TOTAL OVIPOSITIONS SECURED PER DAY FROM 12 ISOLATED FEMALES FOR EACH TEMPERATURE RANGE

Temp., °F.	Days																							Totals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Yellow strain																								
100	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
95	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26
90	290	55	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	345
85	309	45	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	362
80	255	171	67	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	520
75	282	128	107	41	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	564
70	259	118	55	44	57	31	37	11	22	8	16	5	5	2	2	2	–	–	–	–	–	–	–	674
65	227	154	132	57	60	44	44	21	12	9	1	2	4	1	–	–	–	2	–	–	–	–	–	770
60	211	73	243	128	247	67	58	36	50	28	15	17	9	–	–	–	–	–	–	–	–	–	–	1182
55	95	55	47	23	22	19	27	10	27	7	16	10	17	10	11	5	6	7	9	8	2	1	1	435
																								4878
Gray strain																								
100	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
95	78	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	78
90	374	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	374
85	338	123	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	461
80	295	55	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	349
75	275	142	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	432
70	415	80	15	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	543
65	284	67	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	370
60	217	108	20	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	346
55	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
																								2953

No blackened eggs were observed from which *Ephestia* larvae emerged. This was also true of the breeding work done with eggs of the oriental fruit moth. All blackened eggs represented killed host eggs. This is at variance with the findings of Schultze (1926).

The ovipositions of 12 isolated females are taken as representing the average for each race at each of the 10 temperature stations chosen, and to save space these are totalled by days in Tables III and IV.

TABLE IV

SUMMARY OF REARING AT DIFFERENT CONSTANT TEMPERATURES

Temperature, °F.	Oviposition			Length of life, days		
	Average*	Maximum	Minimum	Average*	Maximum	Minimum
Yellow *Trichogramma*						
55	36.2	43	27	23.0	32	9
60	98.4	166	43	15.2	23	4
65	64.1	101	36	12.6	21	2
70	56.1	123	23	8.0	16	2
75	47.0	83	22	4.0	6	2
80	43.3	62	17	3.5	6	2
85	30.2	60	11	1.5	3	1
90	28.8	60	4	1.2	2	1
95	2.1	12	—	—	—	—
100	—	—	—	—	—	—
Gray *Trichogramma*						
55	—	—	—	4.0	6	1
60	29.0	56	2	2.7	4	1
65	30.8	57	18	2.3	3	1
70	45.0	62	22	2.1	5	1
75	35.8	48	12	2.0	3	1
80	29.0	42	13	1.3	2	1
85	38.4	51	20	1.5	2	1
90	32.0	40	22	1.0	2	1
95	5.5	16	—	1.0	1	1
100	—	—	—	—	—	—

A consideration of the relation of the two strains to temperature suggests that the native strain is the better adapted for activity within the lower seasonal temperatures, and the gray strain better suited to the higher mid-summer ranges. The native strain produces its maximum oviposition near 60° F., while the southern strain reaches its greatest efficiency some 10 degrees higher.

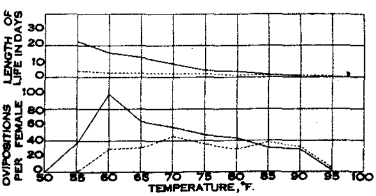

FIG. 6. *Curves showing the average life and average ovipositions of Trichogramma minutum Riley. The upper curves represent the average life of the strains, the lower curves the average ovipositions as taken from 12 isolated females (—— native strain, ... gray strain).*

TABLE V

THE AVERAGE PERCENTAGE OF FEMALES OBTAINED FROM THE INDIVIDUAL REARINGS AT THE DIFFERENT CONSTANT TEMPERATURES

Temp., °F.	55	60	65	70	75	80	85	90
Yellow females, %	62	61	64	73	64	63	65	61
Gray females, %	---	63	66	65	65	70	67	64

No particular trend in the relative numbers of males and females was observed throughout the experimental temperature range.

Storage of Parasitized Host Eggs

During the course of the laboratory work, various lots of parasitized *Ephestia* eggs were placed in cold storage chambers, ranging in temperature from 20 to 50° F. Unfortunately there were no means of determining or stabilizing the moisture content of the apparatus, and it was not possible to take this factor into consideration. Recently deposited parasite eggs remained healthy within the host eggs for a period of three days at 30 to 32° F., but after this time showed a mortality which increased rapidly with the length of storage. Developing parasite larvae within host eggs continued their growth after seven days' storage at the above temperature, but with longer periods showed an increasing mortality. In this case, death appeared to be due to the desiccation of the host eggs which often completely collapsed. More satisfactory results were obtained by allowing the developing parasite to reach the pupal state before placing the host eggs in storage, but it was also found that temperatures ranging between 35 and 45° F. gave much lower mortality. Under these conditions satisfactory emergence was obtained after a storage period of 75 days, although the emerging females laid only half as many eggs as those allowed to develop normally.

Because of the small amount of yolk and the relatively delicate chorion of eggs of *Ephestia kuehniella* Zell. they are not a very satisfactory host for holding *Trichogramma* in storage for long periods of time, and much healthier *Trichogramma* adults were obtained from these eggs when development was merely retarded by rearing at a temperature slightly above 55° F.

Adult Activity

The following observations are recorded as indicating the activity of *Trichogramma* adults. The information was obtained from three main sources: (i) from seven releases of 2500 *Trichogramma;* three of the gray strain and four of the yellow strain (the activity of the parasites was observed throughout the day of the release and the following day, and notes were kept on the rate and type of activity and the weather conditions); (ii) from the observations made during and subsequent to the larger colonizations; (iii) from studies made under controlled conditions in the laboratory.

Considerable difference in the activity and habits of the two strains under consideration were noted. The gray race was more active, took wing more readily, and was more rapid in dispersal. The yellow race moved more consistently by crawling and flights were of shorter duration. Generally, these flights were between nearby leaves. In the absence of host material both

strains were restless and rapidly dispersed. An examination of the foliage of a tree containing only a few eggs 24 hr. after a release of 2500 gray parasites, showed that they had completely disappeared. In another case where numerous eggs had been artificially planted on the foliage, gray *Trichogramma* were present the day following the colonization.

During the summer, the general activity of both strains seemed regulated by the abundance of host material present and the intensity of light.

The influence of light. Light intensity is the most important factor affecting the activity of *Trichogramma* in the field. The insect is positively phototropic and moves toward natural light even in the presence of much stronger artificial light. A knowledge of its activity in different light intensities may be used to advantage in interpreting its behavior on the foliage. Some idea of the insects' reaction to light may be shown by the results of an experiment conducted in an apparatus which allowed a range of temperature from normal (55 to 85° F.) to lethal (115° F.). Native yellow *Trichogramma* were placed in a glass tube four feet long, and allowed freedom in choosing the temperature they preferred. By darkening the tube in the lower ranges of temperature and applying a strong light on the part containing the lethal temperature, it proved possible to attract the insects into the higher temperatures where they died rather than return to the darkened areas.

Parasitized host eggs from which the parasites were due to emerge within an hour were placed outside at dusk and their emergence was thus delayed until sunrise the following morning. As the sun's rays intensified, the parasites rapidly left the host eggs. Under such circumstances the lower night temperatures also assist in retarding the emergence, but light was the important factor since emergence may be delayed several hours at a constant temperature by placing the parasitized eggs in total darkness. This retarding effect of darkness was used to advantage in making the larger liberations. The laboratory breeding of the parasite was so arranged that emergence was due to begin some time during the evening or night before the liberation was desired. The cards containing the parasitized host eggs were sealed into the waxed paper cones and these were hung in the liberation trees during the afternoon previous to normal emergence. The *Trichogramma* adults began to emerge shortly after sunrise the following morning, and were advantageously placed to attack the eggs of the oriental fruit moth deposited on the foliage during the previous evening.

Orchard observations on the activity of the parasite in exposed situations suggest a changed rate of activity when a cloud passes in front of the sun. Ordinarily on peach leaves a less marked reaction is noted. This is probably due to the diffused condition of the light produced by passage through the foliage. The insect does not seem to be so directly stimulated by the varying light intensities when in the body of the tree, and passes readily in all directions on the limbs and foliage, in fact, the greater majority of the parasites are to be found on the under surfaces of the leaves. Its activities in this case are probably influenced by its search for host eggs.

Intensity of light has a stimulating effect on them, which was especially noticeable during the liberation of laboratory-reared material. On dull days the insects rarely took flight unless disturbed, while on bright days adults were often observed to take flight, in fact, this appeared to be the desired means of transportation. The latter was particularly true of the gray race. When moving normally about on the leaves, the flights were of short distances, seldom over seven inches, although often the insects flew much greater distances. *Trichogramma* released in exposed locations reacted very definitely to the light intensity and immediately took flight. They appeared to leap free of the surface on which they were resting and rise some 10 to 16 in. before assuming any definite direction. In the absence of wind they disappeared in all directions.

All the observations indicate greater activity in the gray strain than in the native strain, and a tendency for wider dispersal.

The influence of wind. The direction and velocity of wind is a very important factor in the establishment and distribution of liberated *Trichogramma* within an orchard. The following observations illustrate the influence of air currents, but particularly the combined effects of wind and light intensity. A Petri dish containing 5000 emerged gray *Trichogramma* was placed in the lower unprotected portion of a tree between the larger divisions of the trunk. The top of the dish was partly removed to allow the parasites free access to the tree. With a temperature of 80° F. and alternating periods of sunlight and shade, produced by a cloud passing before the sun, the following observations were made.

When the sunlight was intense, the insects crawled from the dish and, after a short examination of their surroundings, took flight, generally flying upward in an erratic manner. The general direction of the flight seemed dependent upon the velocity of the wind. When a light breeze of one mile or less per hour was blowing, the flight was in a windward direction, probably veering either to the right or left of the wind course. When the velocity reached nearly three miles per hour, the insects were carried along with it. The behavior of the insect previous to, and during the beginning of, flight suggested that it headed into the wind and, when the velocity was greater than the speed of flight, was carried along with it. During the shaded periods the behavior of the insect was varied. On one such occasion a *Trichogramma* was observed to crawl 11 in. along a branch but this individual took wing soon after the appearance of the sun. Within an hour all the parasites had left the dish and none were observed to have crawled to the peach foliage 24 in. away, although *Trichogramma* were subsequently found on this branch. Dispersal from the liberation point was in all directions and the factor influencing the direction of flight appeared to be the direction and velocity of the wind.

The observations made in the orchards where large liberations were placed indicated that the later distribution and amount of oriental fruit moth egg

destruction depended in a large measure on the direction and velocity of the prevailing winds during, and directly following, the emergence of the *Trichogramma* from the laboratory host eggs.

The influence of precipitation. Only one opportunity was given to observe the activity of *Trichogramma* during a rainstorm, and while the foliage was still wet. In 1929 a heavy rainstorm blew up while the parasites were leaving the cones and considerable time was spent watching the reactions of the insects to the rain and the moist conditions of the limbs and foliage.

This was during the liberation of some 500,000 gray *Trichogramma* in a number of orchards. The temperature was 72° F., with little or no wind.

Except during the showers the insects continued to emerge slowly from the cones. Most of the parasites were crawling about on the cones or were perched on the under surfaces where they were protected from the rain. Some few were making their way onto the limbs and could be seen crawling about slowly. A few *Trichogramma* were observed four feet from the liberation cones, but these may have left the cones before the storm, since none were observed to crawl that distance during the period when the tree was very wet. No parasites were seen to take wing, although thousands were within short distances of the cones. Careful notice was taken to see whether the free moisture was detrimental to the *Trichogramma* and whether they became ensnared in the droplets as they do in free moisture in breeding containers in the laboratory. None were observed dead in the free moisture upon the limbs, but several were caught in fine spider's silk stretched between the coarse pieces of bark on the branches.

The observations suggest that rainstorms slow up the distribution of the *Trichogramma* within the tree and throughout the orchards, but otherwise do little damage. Probably, in a heavy storm, a number would suffer mechanical injury, but, on the whole, the parasites appear to be protected by the foliage.

The influence of predators and parasites. Few cases were observed where the adult *Trichogramma* suffered from the activity of enemies. Adults were observed enmeshed in the silken strands placed by spiders, but such cases were scarce, and, as far as the field observations could determine, few were destroyed by predators.

The developing parasites in the host eggs on the leaves were more exposed to attack. This was especially noticeable during 1930 and 1931, when great numbers of chrysopid larvae appeared on the peach foliage and remained throughout the season. A mortality as high as 60% was found in some of the orchards during the second generation of the oriental fruit moth in 1930. These voracious little chrysopid larvae thrust their mouthparts into the host eggs containing the *Trichogramma* larvae or pupae and suck the contents. One chrysopid larva was observed to destroy four eggs in 15 min. Because of the activity of these chrysopid larvae, both the oriental fruit moth and *Trichogramma* were greatly reduced in number.

It is also interesting to note that from 2 to 10% of the stalked eggs of the chrysopids were in turn parasitized by *Trichogramma*. The *Trichogramma* adult ascended the egg stalk and clung to the egg while ovipositing.

During these studies no secondary parasites were reared from parasitized eggs collected in the field.

The influence of artificial control. Observations made in orchards where oil or hydrate of lime was used as a spray or dust indicated that the normal activity of *Trichogramma* was inhibited when these substances were present on the foliage. Controlled experiments in a large glass container in the laboratory confirmed the field observations and showed that 75% more of the untreated eggs were parasitized than those sprayed or dusted. These substances were distasteful to the parasite and acted as a repellent and practically eliminated *Trichogramma* as a factor in egg destruction in such orchards. This probably explains why orchards sprayed or dusted often showed very little more control than untreated orchards.

The Orchard Activity of *Trichogramma* as Determined by Artificially placed Eggs of *Sitotroga Cereallela* Ol.

The many difficulties encountered in determining the actual numbers of eggs of the oriental fruit moth parasitized during any specific period in the experimental orchards suggested the use of artificially placed *Sitotroga* eggs. Their use was based on the assumption that fresh eggs placed in the trees and collected before hatching would give a fairly accurate picture of the activity of the *Trichogramma* during the period these eggs were available as hosts, and would also eliminate many of the factors which distorted the data secured from the examination of naturally oviposited eggs of the oriental fruit moth.

The *Sitotroga* eggs, some 50 in number, were fastened by shellac to small half-inch white tags provided with a string for attachment in the trees. The eggs used were under 12 hr. old and were transported to the orchard in a specially prepared refrigerator. The tags were numbered and securely tied in various places in the trees and accurate information kept as to their distribution. Throughout these studies, covering a period of two years, over 85,000 *Sitotroga* eggs were used.

Several objections were at once apparent to their use: (i) the concentration of the eggs on the card which facilitated parasitism by one female; (ii) the unnatural conditions offered by the cards; (iii) the influence of weather which would be more pronounced on such eggs; (iv) the species of eggs used were of a stored product insect in an unnatural environment; and (v) the eggs used were of the same species as that used for the laboratory breeding and introduces the possibility of host preference. After a consideration of these objections, it was decided to give this method of determining the activity of *Trichogramma* a trial. The results obtained justified their use and much information was obtained which could not otherwise have been secured.

The only strain of *Trichogramma* used in this work was the native yellow strain. For convenience, the results obtained from the various lots of *Sitotroga* eggs are grouped as follows: (i) the relative egg parasitism appearing in untreated orchards and colonized orchards; (ii) observations on the activity of *Trichogramma* in colonization orchards; (iii) comparative records of egg parasitism in *Sitotroga* eggs and natural eggs of the oriental fruit moth in the same orchard; (iv) parasite dispersals from isolated releases within an orchard; and (v) consideration of the possibility of *Trichogramma* showing a preference for the host on which it is reared previous to liberation.

Throughout the experiments the colonization orchards were treated from every third tree in each third row. In this manner there was only one space between any tree and a treated tree. Each group of cards placed in such orchards was exposed to approximately 18,500 *Trichogramma* to the acre, or from 1,600 to 1,700 parasites per release tree.

Results

1. The orchards receiving releases showed a much higher egg parasitism than the untreated orchards examined at the same time. The figures in Table VI illustrate this difference.

TABLE VI

PERCENTAGES OF EGG PARASITISM IN TREATED AND UNTREATED ORCHARDS

—	1	2	3	4	5
Colonization orchards, %	37.5	27.9	27.1	48.7	47.9
Untreated orchards, %	13.5 and 6.4	3.0 and 0.0	8.1	12.1	12.1

No. 1 record was obtained during the period when eggs of the first generation of oriental fruit moth were present, Nos. 2 and 3 during the presence of eggs of the second generation, and Nos. 4 and 5 during the presence of eggs of the third generation.

2. The records covering the activity of *Trichogramma* within the colonization orchards were collected from one-acre plots in which tags containing *Sitotroga* eggs were placed in every tree. The location of the cards was varied in the different trees and accurate record was kept of their location and subsequent history. The percentages of eggs parasitized on the liberation trees as well as those on the non-liberation trees, and the total for the plots are shown in Table VII.

TABLE VII

PERCENTAGES OF EGG PARASITISM ON LIBERATION AND NON-LIBERATION TREES

—	1	2	3	4	5	6	7
Liberation trees	50.6	35.2	57.4	42.7	60.4	64.0	79.3
Non-liberation trees	32.8	27.2	18.5	15.5	20.9	21.3	30.2
Total	36.5	28.6	20.9	23.5	29.3	42.5	35.2

The figures for Plots 1, 2, and 3, were obtained during 1932, and the remainder during 1933. These figures show a decidedly greater efficiency of the *Trichogramma* in the colonization trees, and indicate that for a uniform activity throughout an orchard, releases would have to be made from every tree.

The regional studies within the individual trees showed a general distribution of the parasites with slightly less eggs parasitized in the central part of the tree.

TABLE VIII

PERCENTAGES OF EGG PARASITISM ON NATURAL AND ARTIFICIALLY PLACED EGGS IN THE SAME ORCHARD

Natural eggs of the oriental fruit moth		Artificially placed eggs of *Sitotroga*
Eggs of first generation,	30.4	37.5 and 27.9
Eggs of second generation,	15.6	27.1
Eggs of third generation,	15.1	48.7
Eggs of third generation,	13.9	47.9
Eggs of third generation,	5.9	12.9

3. A comparison of the egg parasitism obtained in the colonization orchards between the artificially placed *Sitotroga* eggs and the natural eggs of the oriental fruit moth showed greater parasitism on the *Sitotroga* eggs. This discrepancy may be a result of a combination of the objections noted against the use of these eggs, or may, especially during the presence of eggs of the second and third generation of the oriental fruit moth, demonstrate the high dilution of natural eggs by old egg remains on the foliage.

The discrepancy increases with the season which suggests that remains of the eggs of the oriental fruit moth on the leaves may greatly influence the records and give a depreciated picture of *Trichogramma* activity.

4. The difficulty encountered in finding orchards where natural *Trichogramma* were entirely absent limited the number of dispersal studies which could be carried out from single releases. Such an orchard was found in 1933 and three releases of 1666, 5000 and 10,000 *Trichogramma* were made in widely separate parts of the orchard. Owing to the limited scope of the study, the records can be considered to indicate only a dispersal trend.

TABLE IX

EXTENT OF EGG PARASITISM OBTAINED IN DISPERSAL STUDIES FROM RELEASES OF DIFFERENT SIZES

Trees on which counts were made	No. of *Trichogramma* released			Trees on which counts were made	No. of *Trichogramma* released		
	1666	5000	10,000		1666	5000	10,000
	Eggs parasitized, %				Eggs parasitized, %		
Release tree	41.2	42.5	61.5	One tree south	0.0	0.0	19.1
One tree north	13.3	30.4	0.0	Two trees south	0.0	24.0	21.2
Two trees north	4.8	2.1	0.0	Three trees south	0.0	0.0	0.0
Three trees north	0.0	0.0	0.0	One tree west	0.0	0.0	12.1
One tree east	0.0	0.0	5.6	Two trees west	18.6	0.0	0.0
Two trees east	0.0	0.0	7.0	Three trees west	0.0	0.0	0.0
Three trees east	0.0	0.0	0.0				

TABLE X

PERCENTAGES OF EGG PARASITISM OBTAINED ON NATURAL EGGS AND ON THE LABORATORY HOST FOLLOWING ORCHARD RELEASES

Natural O.F.M. eggs, %	—	*Sitotroga* eggs, %
First generation		
30.4	Liberation orchard	37.5
15.9	Check	13.5
7.7	Check	6.4
12.2	Check	0.0
Second generation		
13.9	Three parasite generations after release	4.4
15.6	Liberation orchard	27.1
20.0	Check	8.1
Third generation		
12.0	Six parasite generations after release	7.5
15.1	Liberation orchard	48.7
13.9	Liberation orchard	47.9
17.7	Check	0.0

These studies again indicate the increased activity of *Trichogramma* on the liberation trees, with a progressively higher parasitism as the size of the colony is increased. No reasons are apparent for the uneven dispersal of the parasites, since conditions throughout the orchard seemed uniform.

5. Throughout the studies conducted with *Sitotroga* eggs in the colonization orchards and untreated orchards there appeared a tendency for host preference. The newly released *Trichogramma* reared in the laboratory on *Sitotroga* eggs favored the artificially placed eggs, while the natural strain produced a higher egg parasitism on the normally oviposited eggs of the oriental fruit moth. The observations shown in Table X favor this contention.

The data shown in Table X were obtained throughout the season of 1932. In as far as possible the records for the artificially placed eggs and the normal eggs were obtained from the same group of trees. The figures seem to indicate a tendency on the part of *Trichogramma* to parasitize the host species on which it matured. Should this evidence express a true tendency, it assists in explaining many of the unsatisfactory attempts to use this parasite in pest control.

Experimental Observations on Orchard Liberations

Methods of Liberation

The degree of parasitism secured in a particular tree, or even in an orchard, is not only dependent upon the number of parasites liberated and the abundance of the host eggs, but also, in a large measure, upon the time and manner in which the parasites are liberated. Observations made on the natural appearance of the parasite showed the dependence of the parasite on an abundance of host material for successful establishment and multiplication, which indicated that the desirable time to colonize was when eggs were abundant. In 1929 an attempt was made to release the *Trichogramma* two days before the expected peak of eggs for each generation. In 1930 and 1931 parasites of several ages were placed in the trees some two days before the expected peak of oviposition. The ages of the developing parasites within the laboratory host eggs were arranged to give a continuous emergence covering 10 to 14 days. In 1932 and 1933 a continuous emergence of *Trichogramma* was maintained during the various host egg generations.

Several methods were used for the release of the parasites in the orchards. As defects in one method became apparent, new ones were tried until a satisfactory technique of colonization was obtained. The following is a short description of the method considered as most suited to Ontario conditions.

The waxed paper cone type of release proved the most satisfactory. The parasitized host eggs were placed in a waxed paper drinking cone, and the bottom closed with a round card sealed in with a mixture of equal parts of beeswax and parawax. These cones are 3½ in. across the bottom and 5 in. high and, for field use, had a piece of string some 14 in. long threaded through the apex. The string served for attachment to the tree. Any number of *Trichogramma* of various ages could be sealed in the cone. For liberation, the cones were taken to the orchards and tied on the liberation trees, after which a small piece of the apex end was removed. The cones were light and could be easily carried in large numbers. The host eggs were protected both from the elements and most predators. Less than 5% of the cones showed the presence of predators, even after hanging on the trees for several days. All releases were made from this type of container after 1929. In 1932 and 1933 a smaller drinking cone, 2 in. in diameter, was used and attachment to the trees made with fine wire in place of string. This proved even more satisfactory, since the small cones were easier to ship and handle in the orchards.

Experimental Liberations

The following experiments were conducted during the seasons from 1929 to 1933, in the vicinity of St. Davids, Queenston, Niagara-on-the-Lake, and Grimsby. The points chosen each season for the work were in those districts where the infestation of the oriental fruit moth appeared to offer the best conditions for the work.

The work by Mr. C. W. Smith in 1928 showed a general trend in degree of parasitism proportional to the number of *Trichogramma* released, with very little control in the colonization tree, or those in the immediate vicinity, from liberations numbering less than 1000 parasites. His liberations were made from Petri dishes, which probably induced a much more rapid dissemination from the liberation tree than would be the case where the parasites were allowed to emerge from the laboratory host eggs at the point of colonization.

With an extension of the interest in control from the individual tree, and those nearby to the orchard, the rate of dispersal became an important factor, since the range and rapidity of dispersal would govern the number of colonization trees used in any particular area. An effort was made during the course of the work to determine this dispersal rate, which, unfortunately, seemed to vary to a considerable extent with the weather conditions during the emergence period.

Two courses of determining the amount of egg parasitism presented themselves; that of searching out the natural eggs on the foliage and determining the percentage of parasitism from the ratio found; or of placing twigs containing laboratory eggs in the trees and using the number of those attacked

as the index. It was felt that the first method, while much more laborious, represented the more natural and accurate method of determining the degree of egg parasitism, and it was, with one exception, used throughout this part of the study. Unfortunately, egg shells remained on the foliage for long periods, especially if there were no rains, and tended to dilute the counts and give a depreciated idea of the usefulness of the parasite. During 1929, marked shells remained on the foliage as long as seven weeks, while during the season of 1930 some of the shells laid by the overwintering generation of the moth remained all summer. Of 150 eggs deposited on June 11, 65 shells were still present in early September. This objection was somewhat overcome by studying the ovipositing habits of the moth for the successive generations and adapting the tree examinations to their changing habits. Also, the personal element is an important factor in evaluating the results obtained from the tree examinations. The monotony of examining leaf after leaf and the smallness of the eggs, coupled with the presence of old shells and the activity of egg predators, may have influenced the data obtained, but in general these factors tended to give an unfavorable impression of the activity of *Trichogramma*, rather than an exaggeration.

The first group of experiments was designed to show the degree of parasitism obtained on a known number of eggs. The limbs were enclosed in a cage with female moths. After a number of eggs had been oviposited, a liberation of adult yellow *Trichogramma* was made.

TABLE XI

PERCENTAGE OF EGG PARASITISM OBTAINED WITH A KNOWN NUMBER OF *Trichogramma* AND HOST EGGS IN 1930 (YELLOW STRAIN)

No.	No. *Trichogramma* released	Condition of sky	Temp. at time of release, °F.	Mean temp. for day, °F.	Wind velocity	No. host eggs	Total eggs parasitized	Parasitism, %
1	200	Clear	85	79.2	Moderate	77	46	59.7
2	200	Showers	65	69.9	Moderate	68	34	50.0
3	200	Overcast	84	73.8	Moderate	125	52	42.0
4	200	Overcast	87	82.2	Light	170	119	70.0
5	200	Showers	85	77.9	Moderate	150	64	43.0
6	200	Overcast	70	69.0	Light	260	247	95.0
7	200	Clear	78.5	67.6	Moderate	200	122	61.0
8	200	Overcast	81	73.6	Moderate	150	120	80.0
9	200	Showers	85	75.0	Moderate	70	52	75.0

NOTE: *In general the higher percentages of egg parasitism were secured when eggs were more plentiful and the sky overcast.*

The following group of experiments was conducted during 1929 in different orchards. Wherever possible, the liberations were made near the centre of the orchard. The release trees were separated from one another by a one-tree space, and the trees used for dispersal records were two trees removed from the nearest release.

The tree examinations were made from five to eight days following the release. All the unhatched eggs found during the first generation were reared. In the second and third generation counts the fresh eggs, averaging less than 10% of all eggs found, were discarded in figuring the percentages. To include the fresh eggs necessitated rearing all such eggs until they normally hatched or showed pigmentation denoting parasitism, and there was not sufficient time to make these individual observations.

TABLE XII

Trichogramma RELEASES IN 1929 (GRAY STRAIN USED)

No.	1st generation colonization	2nd generation colonization	No. colonization trees	No. trees examined	Host egg infestation	1st generation		2nd generation		3rd generation	
						Eggs found	Parasitism, %	Eggs found	Parasitism, %	Eggs found	Parasitism, %
1	70,000	—	7	11	Light	20	0	331	1.7	882	9.6
2	40,000	80,000	4	8	Heavy	39	2.6	382	7.6	987	66.5
3	—	225,000	20	26	Light	—	—	272	8.9	700	20.1
4	—	80,000	4	8	Heavy	—	—	330	15.1	1344	57.1
5	—	90,000	5	16	Medium	—	—	495	23.3	620	23.8*
6	—	61,000	4	12	Medium	—	—	303	9.1	794	40.0
Check	½ to ¾ mile distant		—	9	Medium	—	—	—	—	820	16.1

* *Fruit picked at time of examination.*
No orchard liberations were made against eggs of the third generation.

The colonization trees and those nearby showed a higher egg parasitism during the egg generation on which the release was made. After one moth generation had passed, the *Trichogramma* became distributed throughout the orchard and the egg destruction on the colonization trees was no higher than on other trees.

The following experiments were conducted in 1930 with both the imported gray and the native yellow races of *Trichogramma*. A uniform number of parasites was used per acre throughout each individual orchard, but varying in the different experiments from 20,000 to 1,000,000 to the acre. No colonizations were made on the eggs of the first generation, since the studies of the previous year seemed to indicate a failure of the *Trichogramma* to become established when host eggs were scarce. The releases were made in 28 orchards covering some 250 acres, and 10,000,000 *Trichogramma* were used in the work. Because of the time required for tree examinations, it was not possible to trace intimately the results of the liberations in each orchard. The course followed was to trace carefully the *Trichogramma* establishment in a few orchards and to confine the examinations in the rest to the final generation of eggs.

The peculiar field conditions of 1930 made it particularly difficult for the parasites to become established and multiply because there was a relatively small increase in the numbers of moth eggs available in the successive generations (see Fig. 3). This condition may have been partly due to the dry

weather during the oviposition period, but in general was caused by the increase in larval parasitism and the great abundance of chrysopid larvae. These voracious larvae became numerous during the second egg generation and remained abundant throughout the season. They were very active on the peach foliage and destroyed large numbers of unhatched and parasitized moth eggs. This introduced a new source of error in interpreting the activity of *Trichogramma*, since most of the eggs were sucked before those parasitized became pigmented. In an effort to reduce this error, the conditions in each experimental orchard were studied and a conservative allowance made to cover this point.

In these experiments 10,000 *Trichogramma* were placed in each cone and the colonization trees were evenly distributed over the orchards.

TABLE XIII

Trichogramma RELEASES IN 1930 (GRAY AND YELLOW STRAINS USED)

No.	*2nd* generation release	*3rd* generation release	No. of *Trichogramma* per acre	Strains used	*Host* infestation	2nd generation			3rd generation		
						No. eggs	Chrysopid injury, %	Eggs parasitized, %	No. eggs	Chrysopid injury, %	Eggs parasitized, %
1			20,000	Yellow	Light	57	37	9.0	—	—	—
	2,030,000	2,030,000	20,000	Yellow	Light	87	50	9.0	—	—	—
2			20,000	Yellow	Medium	115	41	7.4	72	37.0	37.0
	1,600,000	1,600,000	40,000	Yellow	Medium	238	40	16.1	808	31.6	43.7
3	160,000	160,000	40,000	Yellow	Medium	—	—	—	123	11.4	42.0
4	100,000	—	50,000	Yellow	Medium	310	60	4.7	159	24.0	44.0
5	180,000	—	60,000	Yellow	Light	75	58	3.3	—	—	—
6	—	700,000	100,000	Yellow	Light	—	—	—	281	20.0	61.8
7	—	1,000,000	1,000,000	Yellow	Light	—	—	—	124	26.0	63.0
8	*120,000*	—	*20,000*	Gray	*Light*	*99*	*51*	*44.0*	—	—	—*
9	120,000	—	20,000	Gray	Light	85	38	29.0	—	—	—*
10	*200,000*	—	*40,000*	*Gray*	*Light*	196	51	23.3	112	12.4	29.0**
Check	Average of orchards ½ mile to 2½ miles distant					—	—	—	80	24.0	8.9

* *The parasitized eggs collected during the second generation tree examinations yielded gray Trichogramma.*

** *During the last generation collections of parasitized eggs, 90% of the females were of the gray strain.*

At the time of the third generation release, detached twigs containing eggs of the oriental fruit moth obtained in the insectary were hung in two colonization trees and in nearby trees at the different points of the compass.

These eggs were exposed to the first emergents from the cone, which represented one-quarter of the entire number released for this generation. When the twigs were collected, many of the leaves had wilted badly. The results are given in Table XIV.

TABLE XIV

EXTENT OF PARASITISM OBTAINED ON ARTIFICIALLY PLACED EGGS OF THE ORIENTAL FRUIT MOTH

	Liberation trees	One tree removed	Two trees removed	Three trees removed	Four trees removed
No. of eggs	117	68	137	94	22
Extent of parasitism, %	44.6	37.5	37.7	24.3	0.0

The relation found with the artificially placed eggs of the oriental fruit moth was characteristic of the trend of the orchards generally. The greatest extent of egg parasitism was found on the colonization trees and those nearby and gradually decreased farther away.

Egg counts made 100 yards from the No. 7 plot showed a high parasite dispersal and 43% egg parasitism. The fruit examinations in the liberation plot showed 6.6% damage, while the trees where the dispersal egg examinations were made showed 11.6% damage. Fruit examinations made at picking time in experimental orchard No. 6 showed a fruit damage of 14.7%, while a count 150 yards away showed 26.8% fruit damage. The latter count was made in an orchard influenced by *Trichogramma* dispersal from the experimental orchard and cannot, therefore, be considered a reliable check. The average *Trichogramma* parasitism found in orchards in the vicinity of these plots was only 6%, which gives some idea of the absolute value of the liberations.

The experimental releases of 1931 consisted of 3,810,000 laboratory bred *Trichogramma* of the gray and yellow strains, and 5,000,000 of the native dark strain collected from eggs of *Sialis infumata* Newm. in eastern Ontario. The liberations were made in much the same manner as in 1930, except that generally throughout the season the yellow and gray strains were released in alternate cones. This mixing seemed advisable as a result of the laboratory observations on these strains. The native yellows are more active and prolific at lower temperatures (55 to 65° F.) and the imported grays showed greater activity and fertility at some 10° higher. By mixing the strains, it was hoped that advantage would be taken of weather fluctuations. Two orchards (6 and 7, Table XV) were used for pure releases of the yellows and grays. It was hoped that these would act as a check and also help to tie the work up with the previous years. One hundred thousand *Trichogramma*—half gray and half yellow—were obtained by the Dominion Entomologist from Dr. A. W. Morrill, of California, and these were used in a special experiment at Niagara-on-the-Lake to test out the gelatin capsule method for colonization.

Native *Trichogramma* appeared in the orchards (see Fig. 4) much earlier than in previous years and were plentiful throughout the season. Field studies indicated that their natural abundance was due to a large native host supply and migration into the orchards. This natural *Trichogramma* population was sufficiently large to maintain a fairly constant egg parasitism, which was relative to the host abundance in the various orchards, and it will be seen

TABLE XV

Trichogramma RELEASES IN 1931 (THREE STRAINS USED)

No.	2nd generation release	3rd generation release	No. of *Trichogramma* per acre	Strains used	Host infestation	2nd generation			3rd generation		
						No. eggs	Chrysopid injury, %	Eggs parasitized, %	No. eggs	Chrysopid injury, %	Eggs parasitized, %
1		70,000	40,000 50,000	Native dark	Medium	318	30	41.6	109	25	36.8
2	5,000,000	—	40,000 —	Native dark	Light	43	30	33.3	86	25	40.6
3		—	40,000 —	Native dark	Light	120	30	22.5	100	25	31.6
4			40,000 50,000	Mixed yellow and gray	Very light	22	30	19.0	—	—	—
5	1,300,000	2,040,000	40,000 50,000	Mixed yellow and gray	Very light	20	30	0	142	25	32.4
6	—	200,000	— 50,000	Yellow only	Light	—	—	—	116	25	52.2
7	—	200,000	— 50,000	Gray only	Light	—	—	—	76	25	35.5
Check	Average 5 orchards Queenston-St. Davids			Indigenous	Light	—	—	—	302	25	29.9
8	—	50,000	— 50,000	Yellow only	Light	—	—	—	102	25	31.1
9	—	50,000	— 50,000	Gray only	Light	—	—	—	109	25	29.1
Check	Niagara-on-the-Lake		—	Indigenous	Light	—	—	—	50	25	16.6

that the liberated laboratory stock did not greatly increase the numbers of *Trichogramma* already present. Also larval parasites were more active than in any previous year and chrysopid larvae were still destroying large numbers of eggs of the oriental fruit moth. These conditions resulted in a rapidly diminishing egg supply in the orchards after the second egg generation and rendered conditions particularly unsatisfactory for experimental liberations.

The colonizations were made in a continuous block including all the orchards between St. Davids and Queenston. This was done in an effort

to increase uniformly the parasite population over the entire experimental area and decrease the loss in parasite effectiveness by dispersal from the experimental area. As the season advanced it became apparent that the orchards were too widely separated to benefit from inter-dispersal between colonizations. To secure and maintain a high concentration of *Trichogramma* in orchards where eggs are scarce, as in 1931, would probably necessitate extensive parasite releases in areas between the peach orchards.

The releases were made uniform for the entire experimental area, 40,000 *Trichogramma* to the acre on eggs of the second generation and 50,000 *Trichogramma* to the acre on eggs of the third generation. The tree examinations were so arranged that the counts represent the general condition throughout the entire orchard. Not all the orchards received treatment in both generations.

The 70,000 individuals of the native dark strain, released in orchard No. 1 during the third egg generation, were reared in the laboratory. Orchards Nos. 1, 2 and 3 were in the area of heaviest infestation about St. Davids, where native *Trichogramma* were plentiful. Collections of parasitized eggs made during the egg generation of the release showed many eggs attacked by the dark strain, but collections made in the next egg generation yielded only the native yellow strain. Orchards Nos. 8 and 9 were used to test out the capsule method of colonization at Niagara-on-the-Lake. Four capsules, containing 100 parasites each, were placed to a tree throughout the entire plot. An increase in parasitism is noted as compared with the check.

The *Trichogramma* colonizations of 1932 consisted of 7,425,000 laboratory reared parasites of the yellow indigenous strain. This strain appeared normally year after year in the orchards, and even replaced the gray strain as the season advanced in the experimental orchards. Since it seemed much better adapted to Ontario conditions, it was decided to confine the releases to this strain.

The liberations were so arranged that a release cone was placed in each third tree in every third row. This provided 11 cones to the acre, and, with the exception of the first generation liberation, approximately 5000 *Trichogramma* were placed in each cone, thus providing slightly over 50,000 to the acre. The larger of the first generation colonizations was made with the idea of establishing the parasite in the orchard where it might gradually build up its numbers as the season advanced, and in this orchard 3500 *Trichogramma* were placed in each cone with the same number of cones to the acre. This experiment was similar in many respects to the first generation release of 1929, but, as the imported gray strain was used in that year, it seemed advisable to repeat the work with the yellow individuals. Beginning with the second generation, liberations were made with the object of direct control and three sets of cones were hung out during the presence of eggs of the second generation which provided a continuous emergence of parasites from July 4 to August 4. Four groups of cones were put out during the presence of eggs of the third generation, providing continuous emergence from August 16 to September 20.

TABLE XVI

Trichogramma RELEASES IN 1932 (YELLOW STRAIN USED)

No.	1st generation release	2nd generation release	3rd generation release	Host infestation	1st generation		2nd generation		3rd generation	
					No. eggs	Eggs parasitized, %	No. eggs	Eggs parasitized, %	No. eggs	Eggs parasitized, %
1	483,000	—	—	Medium	173	30.4	293	12.3	240	14.6
2	32,000	115,000	230,000	Light	—	—	50	18.2	169	18.3
3	—	249,000	2,250,000	Light	—	—	516	16.1	533	13.9
4	—			Light	—	—	224	6.5	123	15.5
5	—	—	565,000	Light	—	—	—	—	1294	8.9
6	—	—	1,260,000	Light	—	—	—	—	850	4.7
Check	—	—	—	—	39 25	15.9 10.7	55	5.9	50	4.1

The orchards chosen for the studies had sustained considerable injury due to the oriental fruit moth in 1931 and were located near St. Davids and Niagara-on-the-Lake. During 1932 the pest continued to diminish in numbers, principally owing to the activity of larval parasites, and consequently host eggs were scarce. In all the orchards under consideration the *Trichogramma* trend of previous years was apparent. Where host eggs were abundant a higher egg parasitism was obtained with fewer released *Trichogramma*, and where host eggs were scarce it was difficult to increase materially the mortality by large colonies of the parasite.

The *Trichogramma* releases of 1933 consisted of 2,621,000 parasites placed in a large orchard near Grimsby. The colonizations were moved to this new district so that a higher moth infestation might be available for the work since the severity of the damage from the pest was moving westward. The liberation technique developed in 1932 was used and a continuous emergence of parasites was maintained in the orchard between May 26 and June 30. As the season advanced, it became apparent that the orchard was poorly chosen as little injury due to the oriental fruit moth developed. Because of this reason and the need for economy the *Trichogramma* releases were discontinued in the Niagara district.

On the 10 trees examined during the period, only eight eggs of the oriental fruit moth were found of which one contained a developing *Trichogramma*. The figures given for 1933 for liberation orchards in the studies with artificially placed *Sitotroga* eggs were made in this experimental orchard, and show a high parasitism from the released *Trichogramma*.

A review of the data obtained from the orchards during the course of the investigations indicates that a number of factors influence the degree of control secured from *Trichogramma* liberations. The number of parasite releases is not the only item determining the ultimate amount of parasitism; in fact, the time and manner of liberations, the influence of weather conditions

on the strain used, and the abundance of host material may be even more important. In the presence of an abundant host supply fewer parasites will build up a greater extent of egg parasitism than will a substantially larger release where eggs are scarce. This was clearly demonstrated by the results obtained in 1929, as compared to those secured between 1930 and 1933. As the host supply diminished, a correspondingly larger number of *Trichogramma* was required to obtain an equal amount of egg destruction, and finally a point in host scarcity was reached after which even the largest numbers of liberated *Trichogramma* did not materially increase the egg parasitism.

Because of the interaction of the various ecological factors, it is not possible to foretell accurately, at any time in the season, the future abundance of eggs of the oriental fruit moth, or anticipate with any degree of certainty the amount of egg mortality which would result from a colonization of any given size.

Acknowledgments

The field work embodied in these investigations was conducted as part of the biological control program of the Dominion Entomological Branch, under the direction of Mr. A. B. Baird, in charge of the Dominion Parasite Laboratory, Belleville, Ont. The work was initiated by Mr. C. W. Smith, who conducted the field experiments in 1928. Assistance in securing the field records was rendered by various members of the staff, particularly in 1929 by Mr. Harry McGuffin; in 1930 and 1931 by Messrs. L. J. Briand and H. T. Clarke; and in 1932 and 1933 by Mr. H. T. Clarke, and especial thanks are due these men. The writer is also greatly indebted to Mr. George Wishart and Mr. L. R. Finlayson for the production of the laboratory material and its preparation and delivery to the field station. Thanks are due Mr. W. A. Ross and staff of the Dominion Entomological Laboratory at Vineland Station, Ont., and to Mr. G. G. Dustan, of the Ontario Entomological Department, for insectary oviposition records, meteorological records, and other valuable assistance.

The major portion of the laboratory experiments were conducted at the University of Toronto, and the writer wishes to thank Dr. E. M. Walker for kind criticism and advice during this work, and also in the preparation of this paper.

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NOTES ON THE CHLORIDES OF DIMETHYLANHYDRACETONE-BENZIL[1]

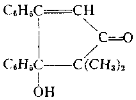
By C. F. H. Allen[2] and E. W. Spanagel[3]

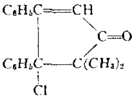
Abstract

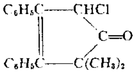
Some of the reactions of the tertiary chloride, obtained as a primary product by the action of thionyl or acetyl chloride on β, β-dimethylanhydracetonebenzil, are described.

Double decomposition reactions with cyclic unsaturated ketones like β, β-dimethylanhydracetonebenzil generally yield isomeric products, the nature of which vary with slight changes in experimental technique; consequently it is not surprising to find that the results obtained by one group of investigators do not agree in all respects with those found by another. In an earlier paper (1) certain reactions of the ketone I were determined as a side-issue, and the complete experimental details omitted for lack of space. Since some of the results were questioned (2), it seemed advisable to re-check them, as well as certain different ones recorded by Burton, Shoppee and Wilson (2).

The principle product from the reaction of I with halogen compounds is the corresponding chloride, II; highest yields are obtained when acetyl chloride is employed (*cf.*, triphenyl carbinol).

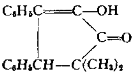

(I)

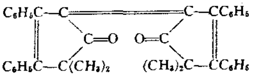

(II)


(III)

This chloride, however, is extremely easily isomerized by basic reagents to a substance which has been assigned the structure III, so the reactions of II may give products that result from either II or III. The isomeric chlorides are readily distinguished by silver nitrate, which gives an immediate precipitate of silver chloride with II, as would be expected of a tertiary chloride.

Hydrolysis of the chloride, II, in similar trials does not always give the same result; the hydroxyketone usually obtained has the structure IV,


(IV)


(V)

[1] *Manuscript received February 2, 1934.*
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but in some instances, small amounts of I have been isolated (best by use of aqueous-alcoholic potassium acetate) and sometimes a yellow bi-molecular product that has been represented as V (2). The substance V is very quickly destroyed by alkalies, but the nature of the reaction is uncertain—it does not give IV, traces of which can be detected by a color reaction with ferric chloride.

It seems advisable to record these results, with experimental details, since a complete investigation in this series is being made elsewhere (2). There seems to be no doubt of the structures of the substances represented by I, II, and IV, but the location of the double linkages in the other compounds of the series, described in this and other papers, cannot be considered as conclusively determined—an admittedly difficult if not impossible task.

Experimental

The chloride, II, was preferably obtained by dissolving the hydroxyketone, I, in a minimum amount of hot acetyl chloride and allowing the solution to evaporate in a current of air. The residue was recrystallized as described previously, the first crop being the desired substance. By the use of thionyl chloride as originally described (1), a reddish gum was obtained; this contained both chlorides II and III, which were separated by fractional crystallization. The isomer III agreed in all respects except melting point (120°C.) with the one described by Burton, Shoppee, and Wilson (they gave 126–127° C.).

An immediate precipitate was formed when six drops of a concentrated aqueous solution of silver nitrate was added to a hot alcoholic solution of the chloride II. A crystalline substance, melting at 120–122° C. and deflagrating when heated on nickel foil, was isolated after filtering the silver chloride, but it was not further investigated.

The action of alkalies on II causes isomerization to III, as well as giving rise to either isomeric hydroxyketone I, IV or the yellow bimolecular product V; while it is easy to secure IV (even by the use of alcoholic-aqueous potassium carbonate), the isolation of I and V must be regarded as fortuitous, because it was not found possible to obtain them at will (except I, by use of potassium acetate), even when reproducing the conditions exactly. Thus, tke original ketone, I, was isolated only twice, in a small yield, by hand-picking the obviously different kinds of crystals; the yellow compound was obtained twice practically quantitatively and once in a small amount, using the same quantities of reactants that usually gave the ketone IV.*

The chloride II (0.1 gm.) was dissolved in 10 cc. of hot methyl alcohol, 10 drops of water added, followed by one drop of concentrated aqueous ammonia. After standing five days two kinds of crystals had formed and were separated mechanically. The prisms were unchanged chloride, m.p.

* *All the reactions described here and previously have been carried out in alcohol containing water, which may account for the fact that different results were obtained by others (2) in very dry alcohol. By alcohol is meant 95% ethanol; all the methyl alcohol was the synthetic variety, used as purchased.*

and mixed m.p., 132° C. and the needles were the isomer, III, m.p. and mixed m.p., 120° C. The chloride II was recovered unchanged from a similar solution containing a drop of concentrated hydrochloric acid.

When equal weights of the chloride, II, and potassium acetate in aqueous methyl alcohol were refluxed a half hour the solution turned orange red and on evaporation left crystals embedded in a gum. The solid was separated and recrystallized twice from methyl alcohol; m.p. 181° C., mixed m.p. 182–183° C. This reaction was checked three times. The gum did not give a color with ferric chloride.

A mixture of 0.13 gm. of the chloride, II, 10 cc. of methyl alcohol, two drops of water, and 0.01 gm. of potassium hydroxide was refluxed 10 min. and allowed to evaporate to crystallization. After filtering and recrystallizing from methyl alcohol (followed by Skelly-Solve *B*, which did not change the melting point) it was identified as the chloride, III, m.p. 120° C., mixed melting point with II, 99–100° C. The same result was obtained by using a mixture of 0.4 gm. of II, 0.5 gm. of potassium hydroxide, two drops of water, and 25 cc. of methyl alcohol, but on refluxing a further 10 min., after adding 1.5 gm. of sodium hydroxide solution (1 cc. = 1 gm.) and filtering the salt that separated, the hydroxyketone, IV, was isolated from the filtrate.

The yellow compound, V, precipitated* when 0.4 gm. of sodium hydroxide solution (1 cc. = 1 gm.), 0.4 gm. of chloride, and 15 cc. of methyl alcohol were refluxed a few minutes. It turned red and melted as described in the literature (2). If the pure yellow compound was refluxed with alkali it slowly dissolved and after 50 min. the solution was practically colorless. It was acidified with acetic acid and a curdy precipitate, that separated on adding water, was separated by filtration. This dissolved readily in the ordinary solvents but separated as a gum. None of the solutions gave a color with ferric chloride.

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* *In two runs only; others gave IV.*

THE OXIDATION OF ALLYL ALCOHOL[1]

By W. H. Hatcher[2] and C. T. Mason[3]

Abstract

The oxidation of allyl alcohol by potassium permanganate in acid solution results in the formation of three molecules of formic acid; up to this point the successive steps involved are favored by acidity of the solution, such that the velocity of the reaction varies directly as the concentration of sulphuric acid. Also the rate of oxidation varies directly as the concentration of the oxidizing mixture. The most rapid part of the reaction is the absorption of the first six equivalents of oxygen, the slowest being that of the last six of the total of 16 concerned. A mechanism is suggested.

Introduction

In previous publications (2, 3) a method was outlined for discovering the mechanism of oxidation of organic compounds by potassium permanganate; this depends on following quantitatively the disappearance with time of the permanganate in dilute sulphuric acid solution in the presence of the substance to be oxidized. If the results be plotted against time for different concentrations of sulphuric acid, which in every case is in excess of that required stoichiometrically, the curves obtained are found to be analyzable into two main forms; one form shows the formation and oxidation of formic acid and the other the presence of a grouping similar in rate of oxidation to oxalic acid. This in brief is the generalization previously formulated.

Procedure

An accurately weighed quantity of the organic substance to be studied is dissolved in water and made up to a definite volume. An aliquot of this solution is then added to a definite volume of an aqueous solution of potassium permanganate and sulphuric acid, the former being in large excess of that required for complete oxidation to carbon dioxide and water of the organic substance, and the sulphuric acid in excess of that required by the permanganate. After standing at a constant temperature for the desired time, reaction is stopped by the addition of standard, dilute hydrogen peroxide. By back titration the volume of standard permanganate used up in oxidation is found.

Experimental

The best available allyl alcohol was used in these experiments but it was found necessary to purify it. This purification briefly consisted of standing in contact with ammoniacal silver nitrate in the absence of light and under an atmosphere of carbon dioxide, and of subsequent distillation in the same atmosphere. It was found that short contact with oxygen produced appre-

[1] *Manuscript received November 27, 1933.*
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ciable quantities of acrolein, hence the necessity for these precautions. The purity of this allyl alcohol was checked by total oxidation by the method of Cameron and McEwan (1).

In addition, the usual care was taken to ensure purity of materials and manipulative accuracy.

Results

For the sake of brevity the results are shown by tabulating the details of typical experiments, as shown in Table I, and by showing in Fig. 1 the curves in which appear the experimental results.

In the following table are given the experiment numbers, and the concentrations of the reagents, — allyl alcohol in gram molecules per litre, and potassium permanganate and sulphuric acid in gram-equivalents per litre. The temperature throughout is 25° C. and the total volume of reacting solution 250 cc.

TABLE I

Molar concentrations of mixtures

Expt. no.	Concentrations		
	Alcohol	Permanganate	*Sulphuric* acid
1	0.0007	0.0011	0.0012
2	0.0007	0.0011	0.0024
3	0.0007	0.0011	0.0036
4	0.0005	0.0011	0.0012
5	0.0005	0.0011	0.0024

The curves shown in Fig. 1 bear the same numbers as the experiments above. Here equivalents of permanganate are plotted against time in minutes.

Complete oxidation of the molecule of allyl alcohol to carbon dioxide and water requires eight atoms of oxygen, *i.e.*, 16 equivalents. Each molecule of formic acid requires two equivalents for complete oxidation. Since hydrogen ion concentration is inimical to the rapid oxidation of formic acid, the rapid formation and slow oxidation of this acid is indicated by the curves tending to parallel the *x*-axis by two equivalents short of the 16 for each such molecule of formic acid.

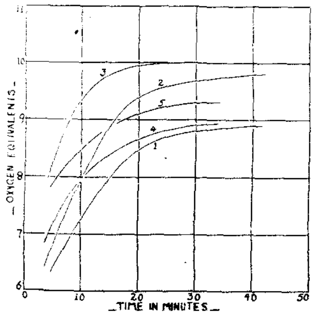

FIG. 1. *The oxidation of allyl alcohol by*

In these curves the maximum is in the vicinity of 10 equivalents, and all are above eight, with two equivalents for each formic acid molecule produced, the difference between 16 and 10 indicates the formation of three molecules of formic acid. The relative positions

of Curves 1, 2 and 3 and also of 4 and 5, show that the course of oxidation of allyl alcohol up to the formation of formic acid, is favored by acidity; this is of course accomplished in several steps not easily identified.

The presence of formic acid is further indicated by the progressive flattening as we proceed from Curves 1 to 3, and from 4 to 5.

From Curves 1, 2, 3 the times to half value—*viz.*, eight equivalents—indicate the velocity of the reaction to vary directly as the concentration of the sulphuric acid; the same holds for Curves 4 and 5. Approximately the same relation holds between the alcohol and the oxidation mixture, as seen by comparing Curves 1 and 4.

Finally, then, up to the formation of three molecules of formic acid, the oxidation of allyl alcohol by acid permanganate belongs to the oxalic acid type. The complete liberation of formic acid then superimposes itself upon the reaction velocity, causing a slow progress beyond 10 oxygen equivalents.

The entire steps may be suggested as follows. Allyl alcohol to glyceric aldehyde, either through aerolein or glycerol, then to glyceric acid; these steps represent the very rapid oxidation with absorption of six equivalents—as seen from the curves. From glyceric acid the steps would proceed to glycollic aldehyde with one formic acid stored up for slow oxidation; thence to glycollic acid which splits with oxidation to form two molecules of formic acid.

Confirmation of these ideas was obtained by examination of a reaction mixture when 10 equivalents of permanganate had been used up. Considerable formic acid was obtained but no other substance could be detected.

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THE SORPTION OF VAPORS BY ALUMINA

II. BENZENE[1]

By L. A. Munro[2] and F. M. G. Johnson[3]

Abstract

The sorption of benzene vapor by alumina gel has been measured over a range of temperature and concentration. It has been found that divergent sorption values obtained with gels differing in residual water approach a constant value when expressed per gram of active alumina. Straight lines are obtained when the logarithm of x/m is plotted against the logarithm of the vapor pressure. Patrick's equation is approximated for lower relative pressures. Comparison of the results obtained with benzene with those previously obtained with water fails to show that hydration or "chemical" forces play any part in the sorption of water by alumina. The gel holds a larger volume of liquid benzene at low partial pressures.

In a previous paper (11) the authors reported the results of an investigation on the sorption of water vapor by alumina gel over a range of temperatures and concentrations. The present paper presents a similar comparative study of the sorption of benzene vapor.

Benzene was chosen for comparison with water because, unlike the latter, it is a non-polar liquid. It is also easily dried and purified (8). Furthermore, it is known that alumina forms hydrates. Fricke and Wever (4) have found that amorphous alumina gel changes gradually at room temperature to hydrargillite, a hydrated form, and if kept at 100° C., the moist gel is converted to micro-crystalline bauxite. Weiser (13) and also Edwards and Tosterund (1) have recently reported X-ray studies of these pseudo-hydrates of alumina. With alumina then, some of the sorbed water may be "combined chemically", that is, there may be an additional force factor operative during the sorption of water vapor. It was thought that the comparison of these vapors might give information regarding the importance of "chemical" forces in sorption processes.

Apparatus and Method

The apparatus and method of study were the same as used in the previous study. Briefly, the method is as follows. Pure air is supplied at a definite rate by an automatic, thermostated Topler pump to a saturator, and thence to a second thermostat containing the alumina tube. The sorption is followed by weighing the alumina at intervals. An arrangement is provided whereby this is effected without removing the sorbent from the thermostat. For diagrams and a detailed study of the accuracy of the apparatus the reader is referred to the previous paper (11).

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From the volume of entering air and the vapor pressure of the benzene, the amount of vapor supplied can be calculated. Curves are obtained by plotting amount supplied against the amount sorbed for each temperature and pressure. These show the efficiency of the alumina. They also give the saturation values for different partial pressures. Isotherms may then be constructed as with a static method of study.

Materials

Gel

The gel was prepared by precipitation from the hot nitrate solution with concentrated ammonium hydroxide, and subsequent dilution and boiling the suspension until it was free of excess ammonia. The precipitated gel was washed free of nitrates and dried at 100° C. for 24 hr. The total water content was determined by ignition overnight.

Before use a weighed amount of gel was activated at 300° C. for one hour. The residual water content of the sorbent was calculated from the loss in weight and the original water content.

Benzene

The benzene used was Baker's best grade. This was further purified by treatment with anhydrous calcium chloride and sodium wire. Before use a portion was siphoned off from the reservoir containing the sodium wire into an all-glass apparatus and the middle portion of the distillate used.

Calculation and Verification of Amount Supplied

The amount of benzene carried over was calculated from the vapor pressures, assuming complete saturation of the air, accurate temperature control, the validity of Dalton's and Avogadro's laws.

To check the amount of benzene supplied, the mixture was pumped into a U-tube of charcoal cooled with liquid air. Results were not satisfactory because the charcoal also sorbed large quantities of air which varied with the level of liquid air in the dewar. Again, the only charcoal then available caused considerable back pressure. Since the amount of benzene carried over depends on the pressure over the liquid in the saturator, it was determined to use a method of checking which avoided sorbents. Determination by condensation from the air stream was not entirely successful. Excellent check results were obtained by a delayed combustion method, in much less time than would be entailed in the preparation of suitable coarse sorbents.

In the latter method a known volume of gas-vapor mixture was pumped into one of a series of containers in an ice bath. The end container was connected to a furnace, the whole system being at 760 mm. pressure. The absorbing train was then attached to the furnace and the benzene carried by oxygen into the furnace in dilute concentration as the bath was allowed to warm up gradually.

A series of seven determinations of the amount supplied per hundred strokes of the pump at 30° C. gave an average of 0.772 gm. The apparatus had been rebuilt since the experiments with water, and the volume of the new pump was 13.42 cc. The theoretical amount is given by the expression $\frac{78}{22400} \times \frac{118}{642} \times \frac{273}{303} \times 1342 = 0.774$ gm. It was therefore assumed that the calculated amount was correct to within 0.5%. This was borne out by the subsequent sorption experiments as shown by the initial values given in Table II (60° C.—Columns 7 and 8; 10 and 11; 50° C.—Columns 1 and 2; 4 and 5).

Expression of Results

Sorption results have been reported in a number of ways. Several investigators have expressed their results as grams per gram of original gel. Most workers, however, have given the amount sorbed per gram of sorbent, which, if a hydrous oxide, contains residual water. It has been shown that sorption varies with the amount of water left in the gel. The writers have found this to be true of the sorption of ether (10) and water vapor by alumina gel. The sorption of benzene at 25° C. by alumina gels differing in residual water content is shown in Fig. 1, data for which are given in Table I (12). In this experiment the gel used was Baker's alumina containing 34.7% water.

TABLE I

THE EFFECT OF RESIDUAL WATER ON THE SORPTION OF BENZENE

No.	Initial weight, gm.	Activation conditions	Water lost on activation, gm.	Grams sorbent	Residual water, %	Weight of benzene sorbed, gm.	Grams active Al_2O_3	x/gm. sorbent	x/gm. active Al_2O_3
1	4.8888	150°—1 hr.	0.0026	4.8862	33.8	0.0016	0.0049	0.33	326.5
9	5.0010	200°—1 hr.	0.1104	4.8906	33.2	0.0135	0.2078	2.76	65.0
2	5.0096	220°—1 hr.	0.1960	4.8136	32.04	0.0198	0.3689	4.11	53.69
10	4.9898	250°—1 hr.	0.4061	4.5837	28.9	0.1470	0.7642	32.07	192.34
B	3.0642	185°—1 hr. 285°—1 hr.	0.3664	2.6978	25.8	0.1532	0.6895	56.8	222.2
3A	2.6000	290°—1 hr.	0.3803	2.2197	23.5	0.1613	0.7173	72.7	225.
D	2.534	295°—1 hr.	0.4012	2.1328	22.4	0.1892	0.7550	88.8	251.
A	3.0636	285°—1 hr.	0.5084	2.5552	21.7	0.2503	0.9567	97.95	262.
C	2.6962	285°—2 hr.	0.4913	2.2049	20.2	0.2284	0.9246	104.	247.
EA	2.6563	160°—3 hr. 300°—1 hr.	0.5434	2.1129	17.9	0.2682	1.0226	127.	262.
16	5.0845	300°—1 hr.	1.1485	3.9360	15.6	0.5494	2.1613	140.	254.
15	2.9896	300°—2 hr.	0.7234	2.2662	13.9	0.3450	1.3614	152.	253.
12	5.0035	350°—1 hr.	1.4058	3.5977	9.18	0.7027	2.6457	195.	266.
G	2.8190	350°—1 hr.	0.7920	2.0270	9.18	0.3938	1.4904	194.	264.
4A	3.0841	380°—1 hr.	0.8980	2.1861	7.88	0.4214	1.690	193.	249.
13	4.8742	385°—1 hr.	1.4760	3.3980	6.33	0.7381	2.7782	217.	266.
5	5.0231	450°—1 hr.	1.5404	3.4827	5.82	0.7567	2.8988	217.	261.
7	4.9715	600°—1 hr.	1.7019	3.2696	0.71	0.8468	3.2026	259.	264.

Sample calculation. Tube G. Original water content of gel, 34.7%. Loss on activation, 0.792 gm. This was associated with $\frac{65.3}{34.7}$ *of 0.792 = 1.49 gm. of active alumina. Amount sorbed per gram of active alumina is 0.3938/1.49 = 264 mgm.*

From this original gel sorbents having different amounts of water were prepared, as shown in the table. The solid line shows the saturation values per gram of "sorbent" plotted against the water content. Apparently measurements with two sorbents even from the *same original gel* are comparable only when the extent of dehydration is the same, if the results are expressed in this manner. Many comparisons made between different sorbents in the literature mean very little.

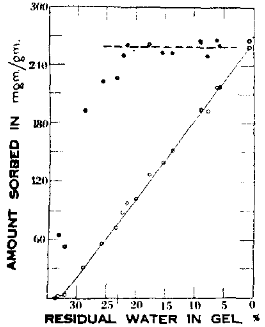

FIG. 1. *Sorption of benzene by alumina gels differing in residual water content. Solid line, sorption values as in mgm./gm. sorbent. Broken line, sorption values as mgm./gm. active alumina.*

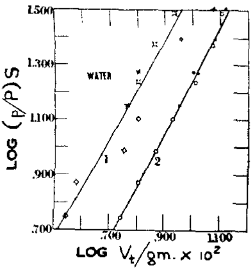

FIG. 2. *Sorption results for water plotted according to Patrick's equation. Curve 1, log (p/P)S against log. vol. of liquid sorbed/100 gm. sorbent. Curve 2, log (p/P)S against log. of liquid sorbed/100 gm. active alumina.*

It will be noted in Table I that only a very small amount of water was given off below 200° C. After the loss of a certain amount of water the sorption of benzene is approximately proportional to the extent of dehydration. This suggests that one of the important activation factors, although certainly not the only one, is the liberation of secondary valences of Al_2O_3, corresponding to additional surfaces or pore space for sorption. The sorption within certain limits depends on the amount of *active alumina*.

The sorbent consists of a certain amount of active alumina with more or less inert or unactivated gel, either original or modified, with probably some water in a new relation to the Al_2O_3. One might represent it as $xAl_2O_3.(yAl_2O_3.nH_2O)zH_2O$, where xAl_2O_3 is the active part and the rest inert, or almost so. The amount of benzene taken up by one gram of "active alumina" calculated for sorbents having from 0.7 to 22% residual water approximates a constant value. This is shown in Table I, or by the broken line in Fig. 1. It means that results for gels of different water content can be put on a common basis.

In any series of sorption experiments, indeed in duplicate determinations, it is of advantage to be able to use tubes of both fresh and reactivated gel. The water content of these may vary somewhat, causing diverging values if expressed per gram of "sorbent". It has been found that results for ether obtained with gels of different water content approach a constant quantity when expressed as amounts per gram of active alumina (10).

Similar results are shown for water in Fig. 2, where they are plotted according to Patrick's equation (7). In Curve 1 the logarithm of the product of the partial pressure and surface tension of the liquid at the sorption temperature, $S(p/P)$, is shown plotted against the logarithm of the liquid volume at that temperature, V_l, per gram of sorbent. Much better agreement is observed in Curve 2, where $S(p/P)$ is plotted against the logarithm of V_l per gram of active alumina (V_l/gm.).

While obviously the method can not be rigorously applied to compare sorption by different types of gel, its value is demonstrated by the three examples above. The results for this paper are therefore expressed in this manner. For comparison with other work the general isotherm is expressed in both ways.

Experimental Results

The results for alumina at 80, 60, 50, and 40° C. and for partial pressures from 74.7 mm. to 147.5 mm. are given in Table II. The curves from the data at 40° C. are shown in Fig. 3. These are typical. The broken line is the total sorption line which the curve follows while the alumina is 100% efficient. The curves for the other temperatures, being very similar, are omitted to save space.

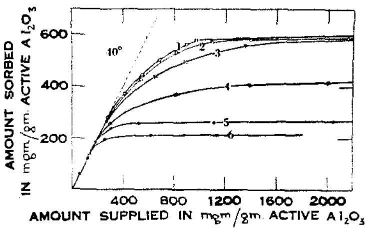

FIG. 3. *Typical sorption curves. Alumina at 40° C. Vapor pressures (mm.):—Curve 1, 178; Curve 2, 167; Curve 3, 147.5; Curve 4, 118.2; Curve 5, 94.7; Curve 6, 74.7.*

Curves 1 and 2 represent the results of experiments in which the vapor pressure was slightly lower than the saturation pressure. Such curves are characteristic. Though the total amount sorbed is not very different from that obtained at lower vapor pressures, the slope is steeper and saturation values are reached more abruptly. This was also noted in the case of water.

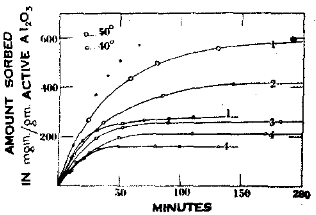

FIG. 4. *Relation between amount sorbed and time. Vapor pressures (mm.): Curve 1, 147.5; Curve 2, 118.0; Curve 3, 94.7; Curve 4, 74.7.*

Fig. 4 shows the relative sorption

TABLE II

SORPTION OF BENZENE BY ALUMINA

A	B	C	A	B	C	A	B	C	A	B	C
80° C.											
Tube 5. P.p., 147.5 mm.			*Tube 5. P.p., 118 mm.*			*Tube 6. P.p., 94.7 mm.*			*Tube 6. P.p., 74.7 mm.*		
176	132	11	125	109	10	59	56.5	6	53.6	50.1	7
353	145	22	250	123	20	154	100.8	16	123	89.8	16
546	145	34	374	124	30	256	103.4	26	191	91.3	25
			574	124	46	394	104	40	268	91.3	35
112	98	7									
225	135	14	123	108	10	49.3	48.5	5	61	58.4	8
353	145	22	222	123	18	118	97.6	12	161	90.5	21
514	145	32	320	125	26	217	103.4	22	421	91.1	55
			480	125	39	394	104	40	*	91.1	
			Tube 6. P.p., 118 mm.			840	104	65			
			118	110							
			236	125							
			413	125							
60° C.											
Tube 3. P.p., 147.5 mm.			*Tube 3. P.p., 118.2 mm.*			*Tube 3. P.p., 94.7 mm.*			*Tube 4. P.p., 74.7 mm.*		
113.7	107.4	10	197	143.4	26	58.9	58.0	10.8	73.3	72.5	14
227.4	150.3	20	343	150	45	176.7	122.8	32.4	133	110.5	32
341	167.1	30	835	150	110	294.5	128.2	54	377	111.6	72
455	168.7	42				825	130.2	151	786	111.4	150
758	168.3	74	304	147	40	1179	129.2	216			
947	168.7	94	835	150	110	1355	129.2	248			
50° C.											
Tube 3. P.p., 147.5 mm.			*Tube 3. P.p., 118.2 mm.*			*Tube 3. P.p., 94.7 mm.*			*Tube 3. P.p., 74.7 mm.*		
105	104	11	120	121.4	16.5	94.1	91.9	18.6	51.7	49.3	10
190	178	20	259	204	36.8	294	167.6	58.0	113.7	110.6	22
305	221	32	435	206	62	470	169.7	93	176	143.1	34
495	250	52	961	208	132	706	169.0	133	258	154.8	50
667	262	70	1678	213	238	1059	169.3	193	414	153.8	80
857	267	90							682	153.8	132
1047	272	110	273	201	30						
1752	273	184	486	207	770				258	152.3	50
22381	272	250	911	209	130				517	154.3	100
			1480	210	211						
			2277	210	325						
			3188	209	455						
			3497	209							
			3796	210							
40° C.											
Tube 4. P.p., 178.5 mm.			*Tube 4. P.p., 167 mm.*			*P.p., 147.5 mm.*			*P.p., 118.2 mm.*		
192	185	14	127	122		133	129	12	127	125	13
411	369	30	280	273	12	1660	548	150	297	258	31
549	443	40	535	429	26	2770	558	250	815	369	85
686	503	50	815	532	48	3650	567	330	1392	407	145
892	565	65	1095	575	75	5310	574	480	1697	409	178
1097†	576	80	1401	578	101	5750	573	520	1870	420	
			1529	583	128				1970	437	
974	580		2802	583	242	267	273	24	2121	433	
1797	583					646	433	58	2970	433	
2620	591					891	497	80	4922	434	
						2785	572	251	*P.p., 94.7 mm.*		
						3890	572	350	87.9	87.8	15
						4467	572	401	187.5	192	32
									305	238	52
									528	258	90
									1067	264	182
									1407	265	240
									2051	266	350
									2638	267	450
									3225	268	550

NOTE:—A = *Amount of benzene supplied in mgm./gm. active* Al_2O_3. B = *Amount of benzene sorbed in mgm./gm. active* Al_2O_3. C = *Time in minutes.* *P.p.* = *Partial pressure.* **Counter stuck.* † *Thermostat slightly high—repeated.*

velocities for different concentrations at 40° C. Two curves for high and low concentrations of vapor at 50° C. are included for comparison.

Efficiency of the Alumina

Efficiency curves may be constructed from the sorption curves. In Fig. 5 the amount taken up per gram with 100% efficiency is related to the concentration of benzene in the vapor-air mixture and to the temperature of the sorbent. These efficiency curves are subject to a relatively large error in transposition from the original sorption curves. They do, however, furnish an approximate estimate of the efficiency for other temperatures and concentrations. They also show the decreasing efficiency with increasing temperature, which becomes more pronounced with high concentrations.

Isotherms

The saturation value for a sorption curve represents the amount of vapor held by the sorbent at a definite equilibrium pressure. Typical isotherms are obtained on plotting these values against the vapor pressure. These are shown in Fig. 6.

If the saturation values for all temperatures are plotted against the relative pressure (p/P), almost all the points fall on one curve, showing that the sorption depends on the ratio p/P and not on the temperature. This will be seen in Fig. 7, data for which are found in Table III. In Curve 1 the saturation values are given as mgm./gm. of sorbent; in Curve 2 as mgm./gm. of active alumina. The agreement is closer in Curve 2.

Owing to differences in the amount of air present at the same relative pressure of benzene for different temperatures, deviations from Trouton's general isotherm would be expected. All equilibrium values will be slightly lower than those obtained by a static method employing the pure vapor unmixed with air.

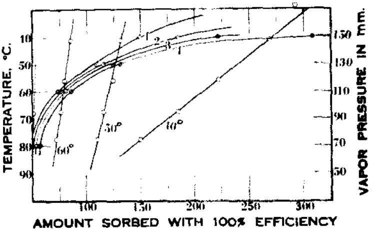

Fig. 5. *Relation of the efficiency of the alumina to temperature and concentration. Concentrations (by volume):—Curve 1, 9.83; Curve 2, 12.5; Curve 3, 15.5; Curve 4, 19.4.*

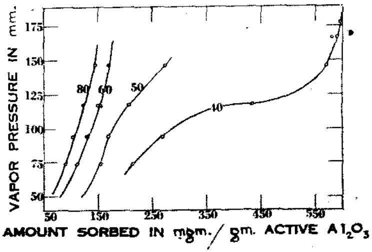

Fig. 6. *Isotherms for 80, 60, 50 and 40° C.*

TABLE III

EQUILIBRIUM VALUES FOR THE GENERAL ISOTHERM AND PATRICK'S EQUATION

Temp., °C.	p/P	Mgm./gm. sorbent	Mgm./gm. active Al_2O_3	V_t in cc./gm. Al_2O_3	$S(p/P)$	Cc. vapor (S.T.P.)/gm. active Al_2O_3
80	0.137	47.6	91.2	0.113	2.79	26.2
	.173	52.6	104.	.128	3.51	30.
	.216	68.2	125.	.153	4.38	35.
	.270	80.3	145.	.179	5.48	36.
60	.192	76.2	111.4	.137	4.23	32.0
	.244	110 and 104	129 and 130	.156	5.36	37.3
	.304	117	150	.184	6.70	44.
	.380	136	169	.202	8.36	48.
50	.278	107	154	.182	6.53	44.2
	.352	134	169	.200	8.27	48.6
	.439	165	212	.248	10.33	60.3
	.548	218	272	.321	12.9	78.1
40	.413	149	214	.251	10.61	61.4
	.523	213	268	.314	13.45	77
	.653	302	433	.509	—	124
	.815	498	572	.670	—	164
	.923	409	583	.683	—	170

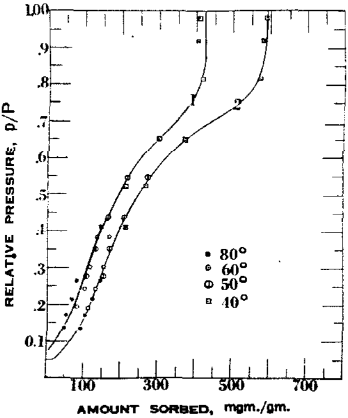

FIG. 7. *General isotherm showing relation of amount sorbed at different temperatures to relative pressure. Curve 1, amount sorbed in mgm./gm. sorbent. Curve 2, amount sorbed in mgm./gm. active alumina.*

When the logarithm of the vapor pressure is plotted against the logarithm of the amount sorbed, straight lines are obtained for the 80 and 60° C. isotherms. With the 50 and 40° C. isotherms, as the vapor pressure approaches the partial pressure of the liquid at the sorption temperature, the curves fall away from straight lines (Fig. 8).

The data for the quantities in Patrick's equation (7) are given in Columns 5 and 6 of Table III. The logarithmic curve in Fig. 8 approximates a line or lines for low partial pressures, but the agreement is not as good as with water.

The change in sorption potential shows that at high relative pressures capillary condensation predominates,

and yet Patrick's equation, derived for pure capillary condensation, does not apply to these very points.

Discussion

As has been pointed out, various hydrates of alumina are known to exist. If, in the case of water, the forces which produce hydration are operative during or immediately after adsorption, one would expect that differences might appear in the general isotherms for water and benzene. These are given in Fig. 9, data for which will be found in Tables III and IV. Equilibrium values are expressed as cubic centimetres of liquid at the temperature of the sorbent in curves marked "1", and as cubic centimetres of vapor reduced to S.T.P., in curves marked "2".

It will be noted that the curves differ considerably. The gel at saturation contains water in greater volume than benzene. It is perhaps surprising to find, however, that, except near saturation, the alumina holds a greater volume of liquid benzene for a given relative pressure. When the vapor pressure is two-thirds of the saturation pressure, the gel has taken up only one-fifth of the saturation amount of water, whereas three-

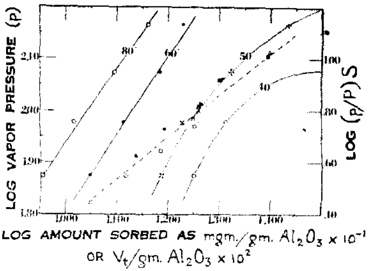

FIG. 8. *Logarithmic curves for* $\frac{x}{m} = K.P.^{\frac{1}{n}}$ *and Patrick's equation. Solid lines, log. vapor pressure against log. mgm./gm. alumina* $\times 10^{-1}$. *Broken line Patrick's equation against log* $(p/P)S$ *against log.* V_t*/gm. alumina* $\times 10^2$.

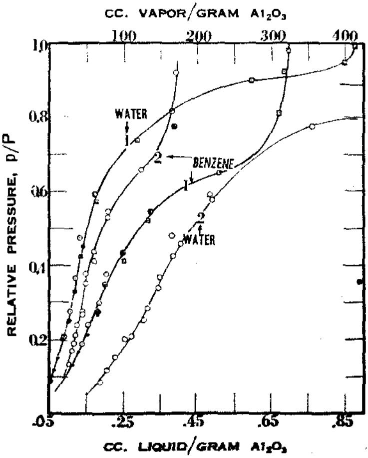

FIG. 9. ***Sorption of water and benzene on alumina—comparison of general isotherms. Curve 1, cc. liquid/100 gm. alumina. Curve 2, cc. vapor/gm. alumina.***

TABLE IV

EQUILIBRIUM VALUES FOR WATER

Temp., °C.	p/P	Vapor cc./gm. Al_2O_3	V_l in cc./gm. Al_2O_3	Temp., °C.	p/P	Vapor cc./gm. Al_2O_3	V_l in cc./gm. Al_2O_3
80	0.089	66.9	0.056	50	0.255	127	0.103
	.118	77.9	.064		.339	146	.118
	.151	89.2	.074		.456	176	.143
	.203	102.	.085		.595	214	.174
					.776	477	.386
60	.211	111.	.090	40	.428	167	.135
	.280	137.	.105		.572	220	.178
	.369	148.	.120		.768	355	.287
	.479	162.	.132		.900	770	.596

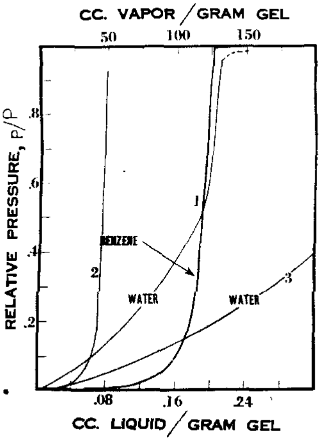

FIG. 10. *Sorption of water and benzene on silica gel—comparison of general isotherms (from Lambert and Foster (5)). Curve 1, Lambert and Foster's curves for benzene and water (Graph V) expressed as cc. liquid/gm. gel. Curves 2 and 3, same results for benzene and water expressed as cc. vapor (S.T.P.)/gm. gel.*

fourths of the total quantity of benzene is held at this relative pressure. One might expect the *positions to be reversed if the* "chemical" or hydration forces played any part in adsorption.

When x/m is expressed as cubic centimetres of vapor at S.T.P. a comparison is obtained of the *relative number of vapor molecules* taken up. It will be seen from the curves marked "2" that the numbers of molecules of water sorbed exceeds that of benzene *over the whole range of partial* pressures investigated.

Recently Lambert and Foster (5) reported very similar results for the sorption of these vapors by silica gel. The data estimated from the initial part of the curves are plotted in Fig. 10. The notations are the same as in Fig. 9.

In a discussion of their results Foster states (3) "When the gel is *half* saturated with water the pressure is $\frac{1}{2}$ of the saturation pressure, as compared with $\frac{1}{30}$ of the saturation pressure for benzene. The forces tending to cause layer adsorption must be weaker for water than for benzene, while the cohesive forces are of the same order of magnitude."

It will be noted however that, if the amounts are calculated in cubic centimetres of vapor, the number of molecules held is practically the same at low relative pressures (0.01–0.04, Curves 2 and 3). These low pressures probably represent true adsorption. Beyond a relative pressure of 0.04 the number of molecules of water taken up by the alumina greatly exceeds the number of molecules of benzene sorbed.

McBain records (6) that with a certain charcoal almost all of the large amount of water sorbed was taken up at a relative pressure of 0.3. With toluene a large percentage of the total amount sorbed was taken up at vapor pressures of a few hundredths of the saturation pressure. Again, the final saturation values for water exceeded those for toluene and other organic solvents, as in the present investigation.

Apparently the larger capacity of alumina for water vapor does not point to the action of hydration forces but rather to the relative size of the water molecule and its polar nature. Again, Milligan (9) reports that the X-ray pattern of the alumina does not change during sorption. There seems, then, to be little necessity for the assumption that hydration or any special "chemical" forces play any marked role in the sorption of water by alumina.

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TESTS FOR MESOTHORIUM IN RADIUM FROM LABINE POINT, GREAT BEAR LAKE[1]

By G. C. Laurence[2] and F. B. Friend

Abstract

A sample containing 50 mgm. of radium bromide, prepared from ore obtained from the Echo Bay deposits in Canada, was tested for mesothorium impurity by the gamma ray absorption method. The material was found to be free from mesothorium within the limits of the experimental error, 0.2% radiation equivalent.

As a confirmatory test a number of samples of the ore were analyzed for thorium in the National Research Laboratories, Ottawa. Quantities of thorium up to 0.07% of the uranium content were found, indicating a possible mesothorium impurity in the finished radium of 0.021% radiation equivalent.

Introduction

In appraising the pitchblende deposits on Great Bear Lake, an important consideration is the possibility of mesothorium in the ore. This isotope of radium is an objectionable impurity because it decays by 10% per year. The purchaser of radium naturally insists that it be practically free from this short lived source of radiation.

Mesothorium is a member of the thorium series and exists in old minerals in concentrations of one part in 2.5 thousand million parts of thorium present. Ore containing one part thorium to 100 parts uranium will yield radium containing a mesothorium impurity of 0.0011% by mass. This corresponds to 0.27% by radiation equivalent, since mesothorium radiates 240 times more strongly than radium.

The impurity of radium is frequently quoted in percentage by mass. The purchaser pays for radiation equivalent and therefore is interested in impurity measured in percentage of radiation equivalent. Care is necessary to avoid confusing these methods of rating impurity when making comparison between ores.

Analyses of the ore showing important mineralogical constituents have been published by Spence (2) and Traill (3) of the Dominion Government Department of Mines. These authors have recorded no thorium in the ore. However, small quantities of radioactive impurities were unimportant in their work, and a special search was not made for them.

Test for Mesothorium in Finished Radium

A physical test for mesothorium in the finished radium bromide extracted from this ore has just been completed in this laboratory. Bothe's (1) method was adopted with some modification, permitting the use of a differential method of measurement and a consequent gain in accuracy. The method makes use of the difference in the coefficients of gamma ray absorption in lead between radium ($\mu = 0.50$ cm.$^{-1}$) and mesothorium ($\mu = 0.62$ cm.$^{-1}$). A

[1] *Manuscript received March 8, 1934.*
Contribution from the National Research Laboratories, Ottawa, Canada.
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preparation containing a fraction η of mesothorium shows an apparent increase in absorption coefficient of η (0.62 cm.$^{-1}$ — 0.50 cm.$^{-1}$) in comparison with radium.

Comparison was made of the radiation from approximately equal quantities of radium recently prepared from the Echo Bay ore and radium* known to be practically free from mesothorium. The radiation from the two differed in intensity by a small amount ΔI_1 when measured through a filtration of 1 cm. of lead, and ΔI_6 when measured through 6 cm. of lead. Ignoring second order differences,

$$5 \text{ cm.}\ \eta\ (0.62\text{–}0.50)\ \text{cm.}^{-1} = \Delta I_6 - \Delta I_1.$$

ΔI_1 and ΔI_6 were observed as shifts in the instrument reading when one sample was quickly replaced by the other. This was accomplished by mounting the two preparations on opposite ends of an arm free to rotate about its centre. Suitably rigid construction and spring catches ensured that the radium samples always returned to the same position. The readings of the instruments were calibrated readily in percentage of the intensity by adding a small known amount of radium.

The ionization chamber was a cubical aluminium box of 18 cm. edge having a central electrode leading to the grid of an electrometer valve which indicated the changes in the ionization current. Direct current valve amplification and a low period milliammeter were used. Suitable grid bias potential and a shunt circuit for a milliammeter in the plate circuit compensated for the effect of the average ionization current so that the instrument indicated the small changes ΔI_1 and ΔI_6 directly.

About 1500 readings of ΔI_1 and ΔI_6 were taken with an equal number of "zero" readings. Corrections were applied to ΔI_1 and ΔI_6 to take into consideration the absorption in the sources themselves which happened to be of different thickness. These amounted to $0.0035 \pm 0.0001\ I_1$ and $0.0020 \pm 0.0001\ I_6$ respectively. The possible inaccuracy in computing these corrections will introduce an error of less than 0.04% in the result.

An advantage of the method is that most instrumental sources of error are eliminated by compensation. The seriousness of the others is easily gauged by experimentally increasing their causes. An uncertainty of 0.1% in the result is due to the difficulty of mounting the two preparations, which were of different shape, at the same average height. The error in the result due to all causes, including the small random fluctuations in the readings, is less than 0.2%. Table I shows the values obtained for $\Delta I_6 - \Delta I_1$.

TABLE I

VALUES OBTAINED FOR $\Delta I_6 - \Delta I_1$

$\Delta I_6 - \Delta I_1$, %	No. of readings on which value is based	Weight
+0.04	600	$\sqrt{2}$
−0.07	300	1
−0.11	300	1
+0.06	300	1

* *The Canadian Standards which were conditioned from two tubes of pure radium chloride prepared from Katanga ore in 1924. One of these has been accepted by the International Radium Commission as Substandard XIII.*

The weighted mean is − 0.1%. The corresponding value of η, the mesothorium impurity is − 0.02%, a value well within the limits of experimental error ± 0.2%. Therefore the mesothorium impurity in the sample of LaBine Point radium does not exceed 0.2% by radiation equivalent. This corresponds to less than 0.0008% by mass.

Thorium Content of the Ore

These results have been confirmed by analyses of the ore for thorium. Four samples were tested in the National Research Laboratories, with the following results:—

Sample 1, of silica type ore*, from No. 2 pit, No. 2 vein, was found to contain 0.045% ThO_2 and 61.4% U_3O_8.

Sample 2, of silica type ore, a composite from No. 1 pit, No. 1 vein, and No. 1 pit, No. 2 vein, contained 0.027% ThO_2 and 57.2% U_3O_8.

Sample 3, of carbonate type ore, from No. 9 pit, No. 2 vein, contained 0.02% ThO_2 and 54.2% U_3O_8.

These three samples were kindly provided by Mr. Spence of the Department of Mines. They had been in his possession for some time before the question of mesothorium impurity had arisen, and therefore may be considered as chosen at random.

Sample 4 was a roasted and milled carbonate type of ore obtained from underground working of the 1200 ft. section, No. 9 to No. 12 pit, No. 2 vein. It was found to contain 0.01% ThO_2 and 30.8% U_3O_8.†

It is calculated from the thorium-uranium ratio of these samples that they would yield finished radium having the following amounts of mesothorium impurity.

TABLE II

Sample no.	Mesothorium impurity, %		Sample no.	Mesothorium impurity, %	
	By radiation equivalent	By mass		By radiation equivalent	By mass
1	0.021	0.00009	3	0.010	0.00004
2	0.013	0.00005	4	0.009	0.00004

Conclusion

The mesothorium impurity in LaBine Point radium, as shown in these investigations, is far too small to be of any practical importance or meet with objection from the most fastidious market.

Acknowledgment

The thanks of the writers are due Mr. C. W. Davis of these laboratories for analyzing a number of samples of the ore for thorium content.

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* *See References (2) and (3) for description of ore and veins.*

† *Sample No. 4 was supplied through the courtesy of Eldorado Gold Mines, Ltd., who also*

THE THEORY OF OPTICAL ABSORPTION IN ALKALI METAL CRYSTALS[1]

By W. H. Watson[2]

Abstract

The experimental results of R. W. Wood are compared with theory using the model of free electrons perturbed by the periodic lattice potential. All relevant data are collected in a table in which it is seen that in sodium, potassium, rubidium and caesium the wave-length of the upper limit of the absorption band in the visible and near ultra-violet is proportional to the square of the lattice constant, while lithium occupies an anomalous position. The facts at present available do not permit a completely definite test of the absolute values of these wave-lengths given by the theory.

Introduction

In the Sommerfeld theory of metals, an electron gas is pictured as moving in the field-free space inside a potential wall. With the aid of certain assumptions regarding the variation of electric potential at the surface of the metal it is possible to give an adequate account of the thermionic emission of electrons from metals. When, however, one attempts to explain the photoelectric effect by means of the same model one finds, as Tamm and Schubin (18) have shown, that there is no mechanism to account for conservation of energy and momentum in individual absorption processes in the body of the metal. The most obvious improvement of model which will remove this difficulty, is made by introducing the periodic variations of potential which must exist in the metal lattice, and by calculating the motion of electrons in this three-dimensional periodic field. This problem has been investigated in the general case, according to the perturbation methods of quantum mechanics by Bloch (1), Morse (10), Peierls (13), Wilson (19), Kronig (5, 6), and in a special one-dimensional case by Strutt (17), Kronig and Penney (9) and by Hill (4). However, even although we have complete spectroscopic knowledge of the atoms which make up the crystal and an accurate model of the crystal structure, in no case of an actual crystal has the exact solution been worked out, nor does it appear to be attainable, on account of the complexity of the calculations.

The most striking result of these new theoretical inquiries consists in this, that the energy levels which can be occupied by electrons moving in any particular direction in the crystal are grouped into bands separated by intervals of disallowed energy values. These regions of disallowed energies occur in the neighborhood of the energy values corresponding to which, for the direction in question, selective reflection of the electrons takes place at successive systems of net planes in the crystal. On account of this distribution of allowed energy levels it follows that the optical absorption spectrum of the metal in the visible and ultra-violet may be made up of alternating

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bands of high and low absorption, and that on the short-wave side of an X-ray absorption edge due to solid metal there should occur fluctuations in the coefficient of absorption extending in the spectrum over some hundreds of volts, which depend on the crystal structure of the metal and are not present in the atomic absorption by the same element in a vapor or gas (7).

Absorption bands in the ultra-violet have been known experimentally for a long time in the case of silver, copper and gold and have received theoretical comment in the light of the above ideas from Fröhlich (2) while the transmission band in silver has been discussed by Schubin (14)*. More recently R. W. Wood (20) has observed that thin films of the alkali metals deposited on quartz at low temperatures are transparent over regions extending from 1860 Å to 2050, 2100, 3150, 3600, and 4400 Å in the five metals lithium, sodium, potassium, rubidium and caesium. He has also shown that this high transparency is accompanied by a low reflecting power.

In this paper the writer has brought together selected data on the alkali metals for the purpose of comparing the facts described by Wood with the theory referred to above. The connection between the theory and Wood's observations has been already indicated by Kronig (8).

We consider the motion of electrons in a cubical box of side Ga where G is an integer and a is the length of the elementary cubic cell of the lattice. In a steady state the equation for electron wave functions independent of the time is

$$\Delta\Psi + \frac{8\pi^2 m}{h^2}(W - V)\Psi = 0 \tag{1}$$

where W is the energy, and $V(x, y, z)$ is the potential energy of the field in which the electron moves. The null value of the energy is so chosen that the mean value of V vanishes. In the case of the alkali metals the lattice is body-centered cubic so $V(x, y, z)$ must have the form

$$V(x, y, z) = \Sigma F(x - n_1 a, y - n_2 a, z - n_3 a) + \Sigma F(x - (n_1 - \tfrac{1}{2})a, y - (n_2 - \tfrac{1}{2})a, z - (n_3 - \tfrac{1}{2})a)$$

summed over all n_1, n_2, n_3 less than G. If we express V in a Fourier series we have

$$V(x, y, z) = \Sigma v(k) e^{\frac{2\pi i}{a}(k \cdot r)} + \Sigma v(k) e^{\frac{2\pi i}{a}(k \cdot r')}$$

Where k stands for the triplet of values k_1, k_2, k_3,

$$(k \cdot r) = k_1 x + k_2 y + k_3 z \ , \qquad (k \cdot r') = k_1 (x - \tfrac{1}{2}a) + k_2 (y - \tfrac{1}{2}a) + k_3 (z - \tfrac{1}{2}a) \ ,$$

and summation is over all triplets (k_1, k_2, k_3) where these are positive or negative integers or zero. Hence

$$V(x, y, z) = \Sigma v(k) e^{\frac{2\pi i}{a}(k \cdot r)} \left(1 + e^{-\pi i (k_1 + k_2 + k_3)}\right)$$

If $(k_1 + k_2 + k_3)$ is an even number

$$V = \Sigma 2v(k)\, e^{\frac{2\pi i}{a}(k \cdot r)} \tag{2}$$

* *Note added March, 7 1934: An important contribution to the experimental study of the optical properties of thin films of silver, copper and gold and other metals has been made by A. Smakula (Z. Physik, 86, 3-4, p. 185, 1933: Physik. Z. 34. p. 788. 1933). Schubin's conclusions are not confirmed.*

and if $(k_1+k_2+k_3)$ is odd the contribution to V vanishes. So we may use Equation (2) to represent V with the condition that $(k_1+k_2+k_3)$ is even.

We require a solution of Equation (1) which satisfies the given boundary condition that the waves are contained in a cube of side Ga. According to Bloch (1) this has the form

$$\Psi_{l_1 l_2 l_3}(x, y, z) = u_{l_1 l_2 l_3}(x, y, z)\, e^{2\pi i (l_1 x + l_2 y + l_3 z)/Ga} \qquad (3)$$

where u is a function which like $V(x, y, z)$ has the period a parallel to the three axes of co-ordinates; l_1, l_2, l_3 can assume all integral values positive or negative and zero. They differ in different states of the motion and can be regarded as quantum numbers. For free electrons $u(x, y, z)$ is constant, and for strongly bound electrons its behavior in the neighborhood of a point in the lattice where there is an atom is the same as the Schrödinger function for the isolated atom. The corresponding energies are given in the case of free electrons by

$$W = \frac{h^2}{8\pi^2 m a^2 G^2}(l_1^2+l_2^2+l_3^2) = \frac{h^2}{8\pi^2 m a^2}(\xi^2+\eta^2+\zeta^2)\,, \quad \xi = \frac{l_1}{G} \text{ etc.} \qquad (4)$$

and in the case of the strongly bound electrons in "zero" approximation, by the energy levels of the isolated atoms. The effect of the periodic field of the lattice regarded as a perturbation is to split each non-degenerate atomic level into $2G^3$ levels (body-centered cube); it is essential for the validity of this method that the energy breadth of this group of levels should be small compared with the interval between neighboring atomic levels. In the limit when G tends to infinity, the effect of the perturbation on the free electron model is to divide (ξ, η, ζ) space into zones within any one of which the energy is a continuous function of ξ, η, ζ; on the other hand W suffers a discontinuous change when we pass from one zone to an adjacent one. Each zone contains $2G^3$ levels.

On the quite general ground of the strong electropositive nature of the alkali metals one expects that the free electron model is the correct one from which to start. There is however more definite quantitative evidence in favor of this model. In the first place, the form of the characteristic soft X-ray spectrum from solid lithium agrees very well with that calculated from the Sommerfeld model (12), whereas in beryllium and other metals with two or more conduction electrons per atom such is not the case (16). Secondly, the electrical conductivities of the alkali metals calculated on the assumption of free electrons are of the correct order of magnitude whereas those of copper, silver and gold differ by at least a factor 10 from the experimental values. Thirdly, Fröhlich (2) has shown that the optical absorption of the alkali metals conforms well with the assumption of free electrons whereas that of silver, copper and gold does not. The essence of his argument is that if n is the refractive index for light of frequency ν, and k is the absorption coefficient

$$nk\nu^3 = K + \frac{e^2}{4\pi m^2}\rho(\nu)\,|p(\nu)|^2 \qquad (5)$$

where K arises from continuous absorption (3) while the second term has a non-zero value only in an absorption band, $\rho(\nu)$ being the density of electrons in the initial state, and for free electrons $|p(\nu)|^2$ is small compared with h^2/a^2. In the absorption bands of Ag and Au this condition is not fulfilled while, in the case of the alkali metals, the scanty data available seem to show that it is. Finally there is the transparency observed by Wood which we shall find fits reasonably well with the perturbed free electron model. Before we discuss this, it is not out of place to point out the importance of optical investigation of the complete series of the alkali metals in connection with the theory of electron motion in crystals. On account of the looseness of electron binding to these metals all energies associated with electron transition in an alkali metal crystal are smaller than the corresponding energies in any other metals and this means that the interesting optical properties are brought into a region of the spectrum where the technical difficulties of observation are less serious, caesium, of course, being the most favorable case. Through the series of alkali metals the lattice constant varies from 3.5 Å to 6.1 Å; this allows a good test of the theory relating optical levels with the size of the lattice cell. Finally, from the point of view of refining the theory, simple electrical models of the alkali atoms can be made so as to allow calculation of the important terms in the Fourier series which represents the periodic potential in the lattice. It must be the aim of any theory of the properties of crystals to join together the evidence from absorption and the emission spectra in the far ultra-violet. The alkali metals offer the best hope of achieving this.

The energy discontinuities, which are introduced when we consider the perturbation of the free electron motion by the lattice field, arise in the second approximation from the removal of the degeneracy which exists for states lying adjacent to the common boundary of two zones in (ξ, η, ζ) space. Kronig (5, 6) has shown that if α, β, γ are any integers then the transition from a state ξ, η, ζ to one ξ', η', ζ' is governed by the selection rule

$$\xi' = \xi \pm 2\pi\alpha, \qquad \eta' = \eta \pm 2\pi\beta, \qquad \zeta' = \zeta \pm 2\pi\gamma \tag{6}$$

in the case of a simple cubic crystal. It is readily seen that in the case of a body-centered cube α, β, γ are restricted by the condition that $\alpha+\beta+\gamma$ is an even number. The most frequent transitions occur for the smallest possible values of α, β, γ. The equation of the boundary in (ξ, η, ζ) space between two zones is

$$\xi'^2 + \eta'^2 + \zeta'^2 = \xi^2 + \eta^2 + \zeta^2 \tag{7}$$

Thus the set of values (α, β, γ) determines a plane in (ξ, η, ζ) space, namely, the locus of states (ξ, η, ζ) whose corresponding electron wave is selectively reflected by the system of net planes in the lattice normal to the direction (α, β, γ). Light is absorbed or emitted only when an electron is transferred from one zone to another.

In our free electron model all those states are occupied for which

$$W(\xi, \eta, \zeta) = \frac{h^2}{\quad} (\xi^2 + \eta^2 + \zeta^2) < W \tag{8}$$

where W_i is the top of the Fermi-Sommerfeld distribution. The smallest light energy which is absorbed by the crystal at 0°*K* is

$$W(\xi-2\pi,\eta-2\pi,\zeta) - W(\xi,\eta,\zeta) \tag{9}$$

where the state (ξ, η, ζ) has the energy W_i. This corresponds to the fact emphasized by Fröhlich (2) that the lower frequency edge of the first absorption band of a crystal corresponds to transitions from the highest possessed energy levels of one zone to the lowest (empty) levels of the next. In this absorption band the highest energy which can be absorbed is given by Equation (9) when (ξ, η, ζ) corresponds to the lowest energy of the Fermi distribution. Now, in actual practice, since there is only one state of zero energy, the frequency for which absorption passes into transparency will correspond to an initial state not exactly at the bottom of the Fermi distribution and will depend on the criterion adopted for determining the high frequency edge of the absorption band. On the other hand by examining the course of the absorption curve in the transition region we should be able to calculate the actual energy limit for the second zone. Experiments are now in progress in this laboratory to investigate this in the case of caesium.

Numerical Facts

Wood's experiments were carried out on films of the order of 500 atoms thick but the crystal structure of such layers should be reasonably independent of the surface on which they are deposited. The writer therefore feels justified in using the data for the solid metal. In Table I all energies are

TABLE I

PHYSICAL CONSTANTS OF CRYSTALS AND ATOMS OF ALKALI METALS

Element	Atomic no.	Density at 20° C. (gm./cc.)	Atomic weight (*A*)	No. of atoms per cc. $n \times 10^{-22}$	$a = \left(\frac{2}{n}\right)^{\frac{1}{3}}$ Å	a (obs.) Å	W_i	Ionization potential	$3^2S_{\frac{1}{2}} - 3^2P_{\frac{1}{2},\frac{3}{2}}$	$3^2S_{\frac{1}{2}} - 4^2P_{\frac{1}{2},\frac{3}{2}}$	Transparency limit (L)	$W_0 = \frac{h^2}{2ma^2}$	$\frac{L}{W_0}$
Li	3	0.53	6.94	4.59	3.51	3.46	4.65	5.36	1.84	3.82	6.03	12.07	0.500
Na	11	0.97	23.0	2.54	4.28	4.24	3.13	5.12	2.10	3.74	5.88	8.13	0.724
K	19	0.86	39.1	1.323	5.32	5.33	2.026	4.32	1.60 1.61	3.05	3.82	5.27	0.747
Rb	37	1.53	85.45	1.078	5.69	5.62	1.766	4.16	1.55 1.58	2.94	3.43	4.59	0.726
Cs	55	1.90	132.8	0.861	6.13	6.03	1.521	3.88	1.38 1.45	2.69 2.71	2.81	3.95	0.711

expressed in equivalent electron volts. The data in Columns 3 and 4 are taken from the International Critical Tables (I. 140) and from them n, the number of atoms per cc. (Column 5), and a (Column 6), the lattice constant in Å, are calculated by the formulas

$$n = \rho / A m_H, \quad a^3 = 2/n \tag{10}$$

where m_H is the mass of the hydrogen atom. In Column 7 are given the experimental values of a taken from Neuburger's (11) recent critical table of crystal data. With the exception of potassium the values are given for 100° K. In the subsequent calculations the values of a in Column 6 have been used. W_i is entered in Column 8 and calculated as follows:

$$W_i = \frac{h^2}{2m}\left(\frac{3n}{8\pi}\right)^{\frac{2}{3}} \qquad \text{(one free electron per atom).}$$

$$= \frac{h^2}{2ma^2}\left(\frac{3}{4\pi}\right)^{\frac{2}{3}}$$

In Columns 9, 10 and 11 are given respectively the ionization potential and first two members of the principal series of the optical spectra of these elements expressed in volts. The upper energy of the absorption band observed by Wood is shown in Column 12 and in Column 13 the value of

$$W_o = h^2/2ma^2 \tag{12}$$

Column 14 shows the ratio of values in Column 12 to those in Column 13. It is strikingly evident that the ratio is sensibly the same for sodium, potassium, rubidium and caesium but is quite different for lithium. It may be that this difference in the behavior of lithium is to be correlated with the fact that, whereas in the other alkali metals W_i is less than the energy of the second member of the principal series (Column 11), in the case of lithium W_i is greater.

Using Equations (9) and (4) we obtain in the first approximation, for the upper limit of the absorption band (that is, the energy difference between the bottom of the Fermi distribution and the top of the second allowed zone)

$$W(2\pi, 2\pi, 0) = 2W_o \tag{13}$$

In the second approximation we have to subtract from this the half-breadth of the forbidden band (6) which is given by $2\,v\,(200)$, the second coefficient in the Fourier series (1). We can obtain a lower limit for this from Wood's observations on caesium which transmits from 4400 to 1860 Å(*i.e.*, from 2.81 to 6.65 volts) and according to Wood appears to become less transparent at the upper limit. This gives a difference of 3.84 volts and therefore a lower limit for $2\,v\,(200) = 1.92$ volts $= 0.485\ W_o$. Hence we have the value 1.515 W_o to compare with Wood's observation of 0.711 W_o. The value of the upper limit of the absorption band for the initial state (ξ_1, η_1, ζ_1) is

$$W'(2\pi, 2\pi, 0) = \frac{W_o}{4\pi^2}\left\{ (2\pi - \xi_1)^2 + (2\pi - \eta_1)^2 + \zeta_1^2 - (\xi_1^2 + \eta_1^2 + \zeta_1^2) \right\} = 2W_o - \frac{W_o(\xi_1 + \eta_1)}{\pi} \tag{14}$$

The energy of the initial state is a minimum for $\xi_1 = \eta_1$ and if the second term of the right-hand member of Equation (14) is to account for $0.8\ W_o\ (1.515-0.711)$

$$\frac{\xi_1}{\pi} = \frac{\eta_1}{\pi} = 0.4$$

and

$$W_1 = 0.08\ W_o \sim 0.2\ W_i$$

since

$$W_i = 0.385\ W_o\ .$$

The corresponding ordinate in the Fermi distribution curve is two-fifths of the maximum ordinate. The correction to $W\ (2\pi, 2\pi, 0)$ required in this way to bring the theory into agreement with Wood's observations thus seems too large. Experiments are necessary to remove doubt on this point, and also to determine the breadth of the transparent region by extending observations into the ultra-violet.

Lithium offers another problem. Here the theory in its simple form appears to fail, but as Wood (20, p. 354) has expressed doubt concerning the position of the lithium limit, perhaps the point should not be pressed at present. Nevertheless it is interesting to point out that the value given by Wood is very close to the first radiation potential observed in the soft X-ray emission spectrum of lithium by Skinner (15) who worked with the metal at a temperature supposedly sufficiently high (150° C.) to remove the regularity of the lattice and therefore all evidence of the lattice level scheme.

In conclusion it may be said that while the present experimental facts are insufficient to give a definite decision, the prediction of the theory regarding the dependence of the upper limit of the absorption band in sodium, potassium, rubidium and caesium on the lattice constant is verified, and a rough approximation is obtained to the actual values of these limits.

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VARIABLE PERSONAL EQUATION OF BISECTION IN PRIMARY TRIANGULATION[1]

By J. L. Rannie[2] and W. M. Dennis[3]

Abstract

The investigation demonstrates that errors of appreciable magnitude may occur in the measurement of angles of primary triangulation by certain observers, owing to variable personal equation of bisection. A simple method of eliminating these errors is described.

Introduction

In many branches of scientific practice it is a well understood fact that various observers have different personal equations of bisection of objects, *i.e.*, one observer may consistently point to the left of the centre while another may point as consistently to the right. It is also well known in observatory practice that the same observer may have different personal equations of bisection on stars of different magnitudes. Observing practices are designed to eliminate personal equation as far as possible, and the test of a good observer is not that he have a small personal equation but that he be consistent in whatever personal equation he possesses.

Application to Triangulation

In the measurement of primary triangulation angles it is taken for granted that the personal equation of bisection is practically the same for all objects sighted on, the angle being assumed to be free from error due to variable personal equation; at least it is considered that any residual error is smaller than the error of the angular measurements due to other causes and is not worth eliminating. This probably is nearly true in the case of most observers, as the years of successful triangulation testify, though in any case, if there is any variable personal equation, an error is introduced into the measurement of angles which might better be removed if it involves no more work—as it does not.

Variable Personal Equation

Occasionally, however, it is found that a very consistent observer has a very different personal equation when pointing, say, on signal lights of different size or intensity. In the case of such an observer the angular measurements may be quite appreciably in error, and the observer himself, or lateral refraction, or atmospherics, or theodolites are likely to receive blame which they do not deserve.

Elimination

While investigating the source of certain discrepancies in angular measurements by different observers on the staff of the Geodetic Survey of Canada,

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Department of the Interior, a case of decided variable personal equation on different lights was discovered in one officer who otherwise was proved to be a most consistent observer. Remedial measures were essential in his case, and it is believed worth while to apply the same remedy to all observers on the ground that even a small source of error which can be easily removed is worth while removing. The remedy consists in fitting a simple reversing prism on the eyepiece end of the telescope and taking half of the observations with the image direct and the other half with the image reversed horizontally —the same method as is employed in all observatories.

Tests of Personal Equation

The apparatus by which the effect was first recognized was simple. Two artificial stars were set up in a dark room about 100 ft. from a solidly mounted theodolite. The star in each case consisted of a very fine hole of nearly 0.01 in. diameter in a thin plate with a small electric lamp behind it, a rheostat being employed to regulate the intensity of the light. A program of measurement of the angle between the two stars was arranged with different intensities of the stars, care being taken in the arrangement of the program to eliminate the effect of any gradual change in the position of the stars.

Test Results

The observer mentioned above as having a different personal equation on different lights made the following typical measurements of the angle between the two stars. In the theodolites used the star or light image is centered between two parallel or slightly converging cross wires. Each of the results is the mean of eight measurements, four clockwise with telescope "direct" and four anticlockwise with telescope "reverse".

TABLE I

ANGLE BETWEEN TWO ARTIFICIAL STARS

Both stars fine and dim	Left star dim, right star bright	Difference	Both stars fine and dim	Left star bright, right star dim	Difference
4° 38′ 39″.88	4° 38′ 40″.20	−0″.32	4° 39′ 55″.28	4° 39′ 53″.58	+1″.70
39″.90	40″.55	−0″.65	54″.80	53″.88	+0″.92
4° 39′ 56″.25	4° 39′ 57″.12	−0″.87	55″.40	54″.72	+0″.68
56″.25	57″.12	−0″.87	55″.70	54″.55	+1″.15
			54″.95	54″.28	+0″.67
			55″.08	54″.35	+0″.73

It is evident from Table I that this observer placed the cross hairs farther to the right with reference to the bright star than he did with reference to the small dim star, the difference being over ¾″ of arc on the average. In other words had he had the same conditions in the field his measurement of the angle would have been almost one second too great or too small.

In order to separate his personal equations of pointing between fine dim and large bright lights, a right angled prism was fitted on the eyepiece end

of the telescope whereby the image was reversed horizontally; a number of pointings and circle readings were made alternately on a light when it was dim and bright, followed by the same number of pointings on the same light when it was dim and bright with the prism removed. Table II shows a sample of the readings taken by this observer.

TABLE II

Determination of personal equation

	Prism on; image reversed	Prism off; image direct	Difference	Mean
Large bright light— Mean of 10 single pointings	48″.44	50″.46	−2″.02	49″.45
Small dim light— Mean of 10 single pointings	51″.11	48″.15	+2″.96	49″.63

The figures under the heading "Difference" are double the personal equation of bisection. Thus the personal equation of this observer on the bright light was −1″.01 and on the dim light +1″.48. Had he been measuring the angle between similar dim and bright lights in the field the measurement would have been 2″.49 in error, a very large error for primary triangulation.

Effects of Introduction of Reversing Prism

To remedy the situation the telescope was fitted with a right-angled prism in front of the eyepiece, the hypotenuse of the prism being parallel to the line of sight (1, p. 147). As the field of view is reversed horizontally in one position of the prism and not when it is revolved 90°, personal equation should be eliminated from the mean of two pointings taken with the prism in the two positions.

TABLE III

Angles between lights *A* and *B*

Image reversed or direct (*R* or *D*)	*A* bright and *B* dim	*A* dim and *B* bright
	4° 23′	4° 23′
R	57″.0	56″.3
D	55″.9	57″.6
R	57″.8	56″.2
D	56″.0	57″.2
R	58″.0	56″.3
D	57″.8	57″.5
R	56″.3	56″.6
D	56″.9	57″.5
Mean	56″.96	56″.90
Mean of 4 measures (Image R)	57″.28	56″.35
Mean of 4 measures (Image D)	56″.65	57″.45

To show the effect of this attachment two sets of horizontal angles were measured by this observer between two lights, *A* and *B*; in the first set the left light, *A*, was fairly bright while the right, *B*, was dim; in the second set the left, *A*, was dim and the right, *B*, bright. First, two measurements were made on the first set, then four on the second set, then four on the first set, etc., ending with two readings on the first set to compensate for any gradual change in the positions of the lights. Alternate measurements were made with "image reversed horizontally" and "image direct horizontally". Each measurement

was the mean of two rounds, one clockwise with telescope "direct" followed by an anticlockwise round with telescope "reversed". Table III exemplifies the results obtained.

The effect of varying personal equation of bisection is evident in the set in Table III; also, any error due to it is eliminated by reversing the image, since the means of the two sets (56″.96 and 56″.90) are almost identical.

Lights of such different intensity as were used in some of the above tests are seldom met in actual practice, so that errors of the large amounts indicated in some of the tests are not probable. In addition it is most likely that few observers exhibit such large changes in their personal equation of pointing as are indicated above. At the same time much greater variations in the appearance of lights occur in field practice than it is reasonable to try to reproduce in a laboratory test and, as the personal equation varies with the aspect of the lights or objects sighted on, it is impossible to predict what magnitude of errors may or may not be introduced into the field work as a result of varying personal equation.

Varying Personal Equation when Sighting on Collimators

One might anticipate that angles measured between collimators containing cross wires at their foci would not be seriously in error owing to changing personal equation. Table IV shows that even the small differences between the appearance of the marks in individual collimators may produce changes which are important.

TABLE IV

DIFFERENCE BETWEEN ANGLE MEASUREMENTS BY TWO OBSERVERS ON FOUR COLLIMATORS *A*, *B*, *C* AND *D*

Angle	No. of measurements by each observer	Average difference between observers	No. of plus differences	No. of minus differences
A to *B*	14	+0″.61	13	1
B to *C*	12	+0″.04	6	6
C to *D*	12	−1″.49	1	11
D to *A*	18	+0″.60	15	3

Each of the measurements noted in Column 2, Table IV, was the mean of four rounds, two clockwise with telescope "direct" and two counter clockwise with telescope "reverse". Each of the angles was therefore measured with more than "primary triangulation" precision. It is believed that the probable accidental error of the total measurement of each angle between collimators by each observer separately was less than ±0″.1 of arc. In this case the difference between the measurements by the two observers of the angles *A* to *B* and *D* to *A* was at least six times the probable error of each observer's total measurement while the difference of the angle *C* to *D* was at least 15 times the probable error.

Varying Personal Equation under Field Conditions

To determine what differences, if any, might be expected under field conditions a set of readings was taken by the previously mentioned observer on four field lights from triangulation station Ottawa. The character of the four lights was intentionally made different; those on Duntile and Museum were apparently normal field lights; that on Jackson was made small and faint, a frequent field condition; that on Billings was made as large and bright as is ever encountered under field conditions. Table V gives the angles to the various stations from the initial, Duntile.

TABLE V

ANGLES BETWEEN FIELD LIGHTS

Station	Duntile	Jackson		Museum		Billings	
Light	Light normal	Light small and faint		Light normal		Light large and bright	
Image		Direct	Reversed	Direct	Reversed	Direct	Reversed
	0° 00′	153° 07′		174° 56′		233° 15′	
	0″	17″.1	16″.3	50″.0	49″.7	50″.8	48″.3
		15″.8	17″.0	50″.3	49″.4	51″.4	49″.5
		16″.0	17″.3	50″.0	49″.0	50″.1	46″.5
		14″.6	16″.7	49″.2	48″.8	49″.5	49″.7
		15″.8	17″.4	49″.2*	49″.4*	49″.0	49″.1
		16″.2	16″.4	49″.3*	48″.7*	51″.2	48″.4
		14″.8	15″.6	48″.1*	49.″7*	50″.7	47″.9
		14″.8	16″.1	48″.5*	47″.5*	51″.2	46″.4
Mean	0″	15″.64	16″.60	49″.32	49″.02	50″.49	48″.22
Grand mean		16″.12		49″.17		49″.35	

**In the last half of the set the appearance of Museum light changed owing to drifting smoke.*

The effect of varying personal equation of bisection is evident in the set in Table V. From what has been said previously it is not surprising that the sign of the personal equation changes on Jackson (faint) and Billings (bright). It is, perhaps, of greater significance that there is strong evidence of varying personal equation even in the measurement of the angle between the two apparently normal lights Duntile and Museum.

It is to be borne in mind that the above readings were taken by the engineer who was previously mentioned as being a very consistent observer but who had shown evidence of having a large varying personal equation. Other observers have not shown such large variations in laboratory tests.

In a subsequent extended field test, strong evidence existed that angular errors, by various observers, as large as half a second of arc were eliminated by the use of a reversing prism on the telescope.

IMPROVING THE PERFORMANCE OF PRIMARY TRIANGULATION THEODOLITES AS A RESULT OF LABORATORY TESTS[1]

By J. L. Rannie[2] and W. M. Dennis[3]

Abstract

The lighter types of geodetic theodolites developed during the past decade have permitted considerable economy to be effected in field operations, but have introduced problems of their own. A systematic investigation of one make of these theodolites has been concluded by the Geodetic Survey of Canada in co-operation with the National Research Council and has resulted, first, in the development of a form of test to ascertain the existence of certain errors and, second, in the correction of these errors by improvements to the axis system of the theodolites.

Preliminary

The Wild Precision Theodolite

This lightweight type of primary triangulation theodolite has been used since 1928 by the Geodetic Survey of Canada, Department of the Interior. One of these instruments is illustrated elsewhere (2, Fig. 3). It is noteworthy for the ingenuity and compactness of its design; the circles are of glass and are read through a microscope parallel and close to the telescope. By the adoption of these theodolites considerable economy has been possible in field work owing to the convenience and speed of operation and to the relative ease of transport, particularly in rough country.

Field Experiences

Naturally an innovation is the subject of suspicion until it justifies itself, and a careful check was kept of the field performance of the Wild instruments. There are difficulties in this course owing to the presence of so many sources of error external to the instrument, mainly atmospheric ones. In all cases, however, the individual readings of the Wild theodolites agreed as well among themselves as did those of the older instruments, indicating that accidental errors such as telescope pointing, micrometer reading and circle graduation errors were certainly not excessive. Furthermore, during at least one field season the triangle misclosures, side equation tests, length and azimuth misclosures were all well within the tolerances allowed. While in other cases an unusually large number of stations had to be re-occupied to obtain the required precision, it could not be said definitely that the Wild theodolite was the seat of the trouble.

Before the investigation described below was begun there were only two disquieting features noticed in the Wild theodolites, though in neither case was their exact relation to angular errors fully appreciated. One was that the alidade axis of several theodolites had developed tightness during service and these axes had had to be lapped to remove this. The other feature was "creep" about which more is said later. This was found to a greater or less

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degree in several of the 10 Wild theodolites in the possession of the Geodetic Survey, and naturally gave rise to the feeling that all was not as it should be. Consequently means were sought to ascertain whether there were any sources of error inherent in this particular form of theodolite.

Outdoor Test

Tests of telescopes, circles and other features were made from time to time, but yielded no indication of anything being wrong. Consequently an endeavor was made to develop a test that would indicate the minute differences of the order anticipated between different instruments.

First, a field test was made in which theodolites of different types were used to measure angles from a single station. In this test Wild theodolite No. 558* was compared with two 12-in. instruments of conventional design which had given satisfactory field service over a period of years. The results indicated that No. 558 had a lower grade of performance than the older instruments.

Laboratory Test

Winter having set in attention was given to laboratory tests. In these it is possible to obtain better control of the conditions and generally more easily isolate the effect of any one source of error. Through the co-operation of the Division of Physics and Engineering of the National Research Council, the tests were accordingly transferred to the Physics Laboratory of the Council, using the collimator system established there for surveying instrument standardization.

The apparatus is housed in a sub-basement room where the temperature rarely changes by as much as 1° F. during a day. Fig. 1 is a diagram showing the location of the collimators. These are supported on concrete piers and an adjustable heavy cast iron stand supports the theodolite. During the tests the collimators were covered by cloths, and reasonable precautions were taken to avoid errors due to the heat of the observers' bodies and other disturbing elements. The collimators were found to retain their angular spacing in a very satisfactory manner, but as a further safeguard the test program was so arranged that the effect of any regular movement of a collimator would be compensated in computing the final results.

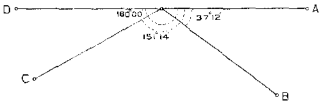

FIG. 1. *Spacing of collimators used in laboratory tests.*

Test Program

In developing a test program it is necessary to evaluate not only accidental errors but systematic errors, and the latter may be much the more important

* *Individual instruments are given their Geodetic Survey numbers.*

and difficult to discover. The former may be evaluated by a comparison of probable errors derived from independent tests of various types of theodolites, and if tests of several instruments which have given satisfactory field performances over a period of years be used as a standard, the capabilities of any other types can be determined by comparison with the standard, as far as accidental errors are concerned.

There is danger, however, that this class of test will sometimes fail to reveal systematic errors, since these errors are liable to repeat themselves and will not be indicated as large residuals. Systematic errors are more likely to be disclosed by a comparison of probable errors derived from an actual comparison of the measurements of the same angles by different types of theodolites, since it is improbable that these errors will be duplicated in the different types. By treating all errors as accidental in calculating probable errors of measurement, the existence of systematic errors is likely to be revealed by a large increase in the probable errors so calculated over those relating to accidental errors.

The ideal program of test should be capable of having the results of each instrument reduced independently and also in combination with others.

With the collimator equipment employed it was found that minute errors in focusing collimators and theodolites, together with very small differences of centering of different theodolites, produced unexpectedly large differences in the measured values of the angles, so that the laboratory test had to be confined to a comparison of independent tests of different theodolites as noted above. In this test systematic errors could be revealed only by varying the conditions which produced them in different sections of the test so that these errors appeared in the form of large residuals which, when used in formulas for accidental errors, produced large probable errors. This necessitated a *prior* knowledge of the existence of these systematic errors so that the form of the test could be designed to reveal them.

The full test program for a theodolite comprised four independent parts, so arranged that each part could be completed in from three to four hours. The method of angle measurement was as follows:—

A round of telescope pointings and circle readings with telescope "direct" was made on successive collimators in a clockwise direction, closing on the selected initial, followed by a corresponding round in a counterclockwise

TABLE I

HORIZONTAL CIRCLE AND MICROMETER DRUM READINGS WHEN THE TELESCOPE IS DIRECTED ON THE INITIAL COLLIMATOR FOR DIFFERENT "POSITIONS"

Position no.	Circle	Drum	Position no.	Circle	Drum	Position no.	Circle	Drum
I	0° 00′	10	VII	112° 00′	35	XIII	34° 00′	10
II	45° 00′	25	VIII	157° 00′	50	XIV	79° 00′	25
III	90° 00′	35	IX	11° 00′	10	XV	124° 00′	35
IV	*135° 00′*	*50*	*X*	*56° 00′*	*25*	*XVI*	*169° 00′*	*50*
V	22° 00′	10	XI	101° 00′	35			
VI	67° 00′	25	XII	146° 00′	50			

NOTE:—*These are the settings used for the Wild theodolite. Theodolites with other dispositions of micrometers, graduations and drum readings have appropriate settings.*

direction with telescope "reverse", *i.e.*, after transiting the telescope. This is called a "position". In the program outlined in Table I "positions I, II, III–XVI" refer to the setting of the circle when the telescope was pointed on the collimator chosen as the initial, as given in Table I.

Part I of the full test program is shown in Table II.

TABLE II

PART I OF THE FULL TEST PROGRAM

Collimators pointed on in the order	Position	Observers
A B C D A	I–IV	*A* and *B*
B C D B	I–IV	*A* and *B*
C D C	I–IV	*A* and *B*
C D C	V–VIII	*A* and *B*
B C D B	V–VIII	*A* and *B*
A B C D A	V–VIII	*A* and *B*

The other three parts have only slight variations from the above. Part 2 starts on collimator *C* instead of *A*, and the circle is read on positions IX to XVI instead of I to VIII; Part 3 begins on collimator *B*, and the circle is read on positions I to VIII; Part 4 begins on collimator *D*, and the circle is read on positions IX to XVI.

An example is given in Table III of the reduced readings obtained in one part of the test program (Part 1; Theodolite No. 1).

TABLE III

REDUCED READINGS OBTAINED IN ONE PART OF THE TEST PROGRAM

Observer	Position	*A*	*B*	*C*	*D*	Closure
		0° 00′	37° 11′	151° 13′	179° 59′	359° 59′
E.M.M.	I	00″.0	56″.5	42″.6	35″.0	59″.5
E.M.M.	II		56″.9	43″.0	34″.9	59″.5
G.F.D.	III		54″.9	40″.8	34″.1	59″.9
G.F.D.	IV		54″.7	41″.3	33″.0	59″.6
E.M.M.	V		54″.4	41″.3	33″.9	60″.1
E.M.M.	VI		57″.5	42″.5	34″.7	60″.2
G.F.D.	VII		58″.0	41″.5	34″.5	60″.9
G.F.D.	VIII		55″.2	39″.4	34″.2	60″.3
Mean		00″.00	56″.01	41″.55	34″.29	60″.00
Mean, corrected for closure			56″.01	41″.55	34″.29	
			0° 00′	114° 01′	142° 47′	359° 59′
E.M.M.	I		00″.0	46″.6	40″.1	60″.6
E.M.M.	II			46″.3	38″.9	59″.5
G.F.D.	III			44″.7	37″.2	59″.5
G.F.D.	IV			43″.9	37″.1	59″.5
E.M.M.	V			46″.0	38″.4	59″.9
E.M.M.	VI			45″.9	38″.2	59″.4
G.F.D.	VII			44″.5	39″.6	60″.6
G.F.D.	VIII			45″.6	38″.2	61″.0
Mean			00″.00	45″.44	38″.46	60″.00
Mean, corrected for closure				45″.44	38″.46	
				0° 00′	28° 45′	359° 59′
E.M.M.	I			00″.0	53″.0	59″.9
E.M.M.	II				53″.2	59″.7
G.F.D.	III				51″.8	59″.4
G.F.D.	IV				52″.9	60″.5
E.M.M.	V				51″.6	60″.2
E.M.M.	VI				53″.5	60″.7
G.F.D.	VII				53″.3	60″.5
G.F.D.	VIII				52″.4	61″.0
Mean				00″.00	52″.71	60″.24
Mean, corrected for closure					52″.59	

NOTE:—*The readings of this set were not taken in the above order, but have been rearranged for ease of computation. The order of taking the readings is indicated in Table II.*

The method of adjustment of the sets is outlined elsewhere (2). As an example the result of the adjustment of the set in Table III is given in Table IV,

TABLE IV

RESULT OF THE ADJUSTMENT OF THE SET IN TABLE III

Angle	Adjusted	Measured	v	Angle	Adjusted	Measured	v
A–B	55″.99	56″.01	−0″.02	B–C	45″.57	45″.44	+0″.13
A–C	41″.56	41″.55	+0″.01	B–D	38″.31	38″.46	−0″.15
A–D	34″.30	34″.29	+0″.01	C–D	52″.74	52″.59	+0″.15
							$\Sigma v^2$0″.0625

from which is obtained

$$r_8 = \pm 0.6745\sqrt{\frac{\Sigma v^2}{n-q}} = \pm 0.6745\sqrt{\frac{0.0625}{6-3}}$$

$$r_{16} = \frac{r_8}{\sqrt{2}} = \pm 0''.069$$

which gives the probable error of the measurement of an angle in 8 and 16 circle positions. The latter is the form used throughout this paper.

As indicated on page 349 the full test on each theodolite was divided into four parts, each similar to the above, and the mean of the four probable errors is considered the value for that full test.

Standard Probable Error

The results of the full test program described previously with three theodolites of older patterns which had given satisfactory results over a period of years, and with one Wild theodolite, are given in Table V.

TABLE V

MEAN PROBABLE ERRORS OBTAINED WITH THREE THEODOLITES OF OLDER PATTERNS AND ONE WILD THEODOLITE

Part	Two-micrometer theodolite No. 1	Three-micrometer theodolite No. 2	Two-micrometer theodolite No. 3	Wild theodolite No. 558
1	±0″.069	±0″.114	±0″.112	±0″.113
2	±0″.106	±0″.090	±0″.046	±0″.101
3	±0″.035	±0″.142 ±0″.058	—	±0″.105
4	±0″.120	±0″.092	—	±0″.105
Mean	±0″.083	±0″.099	±0″.079	±0″.106

As a result of this and other similar tests a probable error of ±0″.1 was decided on as a standard of accidental errors by which the performance of various theodolites could be judged, when subjected to the full test program on collimators as outlined above.

The usefulness of the probable error $\pm 0''.1$ as a standard of comparison depends on the employment of the same laboratory equipment and observational program throughout the tests and on calculating the probable errors on the same basis in all cases. Probable errors have, in this investigation, been based on a comparison of the *averages* of eight measurements of various angles, and no account has been taken of the divergence of individual measurements. Anyone making similar tests would have to develop his own standard based on his instrumental equipment, observational program and basis of computing probable errors.

It is to be stressed in regard to the above program that the probable error is simply a measure of the *agreement* of average measurements of angles. It is not necessarily a measure of the *accuracy* of the angle measurement. If, for example, the readings on a certain collimator contained any systematic error throughout the program the probable error would be no larger than if the systematic error did not exist, but the angles as measured would be in error by that constant amount. Only by changing the conditions in different sections of the test, so that any systematic error appears at different places in the various sections, is that systematic error revealed by means of a larger probable error.

This is the reason why Wild theodolite No. 558 shows up well in comparison with others in Table V whereas, as mentioned on page 348, the outdoor test showed it to be inferior. In later variations of the tests systematic errors were indicated in this theodolite (see Table IX).

Possible Sources of Error

When a few test results were obtained it was decided to take stock of the situation. The facts given below were not all arrived at in the order shown, but can be appreciated better when set out in sequence.

First of all the following definite information had been acquired:—

(i) Creep existed to a greater or less degree in six of the 10 Wild theodolites. Creep was the name given to the following characteristic. If telescope pointings are made after the theodolite has stood for some time with the telescope directed approximately on an object, the pointing will not change. If, however, the telescope is pointed and the circle read immediately after the alidade has been rotated through 360°, pointings and circle readings may creep for a period perhaps as long as eight minutes and by various amounts up to six or seven seconds of arc.

Creep varied greatly in different theodolites. In some it was consistent and unmistakable, sometimes it occurred only at certain circle readings, in one or two it was found only after the telescope had been transited, while in other cases it was present but difficult to detect. One theodolite, previously free from creep, developed it after the alidade axis had been lapped to improve the ease of rotation. It was evidently caused by a lateral movement of the alidade within the limits of the upper locating bearing of the alidade axis, owing to some imperfection of the axis system—alidade or telescope, or both.

(ii) Conflicting results were obtained in the outdoor and laboratory tests with Wild theodolite No. 558. The former indicated a definitely lower precision for No. 558 in comparison with older types, whereas the latter test (see Table V) had shown very little, if any, inferiority for this theodolite. The latter test apparently failed to reveal whatever faults existed.

(iii) The alidade axis of some of the Wild theodolites had developed tightness during service, and these axes had had to be lapped to eliminate this. This fact definitely indicated warp of the metal.

The Wild theodolite is constructed with cylindrical steel telescope and alidade axes, and these parts are fitted with very small tolerances, the fits and workmanship approximating to the type of work usual in gauges and machinists' tools.

The following suggestions were therefore brought forward:—

(a) No matter how nearly perfect the workmanship there is always an error due to the actual forms of cylinders and surfaces not exactly conforming to the ideal geometrical shapes they are designed to have.

(b) Many metals, particularly in the first few years after they have been heated or deformed in mechanical operations are subject to dimensional changes. This fact is well known in workshops, where, despite elaborate precautions, it often leads to trouble. Steel gauges show secular variations (4), and even the bronze Imperial standards of length have shown progressive changes (3).

(c) As a corollary to (a) and (b), if the tolerances between parts that move relatively to one another are small it is quite possible to see how defects may develop in an instrument which is free from them at the time of construction. This is particularly the case with cylindrical fits having very small tolerances.

Strain

As both the horizontal and the vertical axes of the Wild theodolite fall within this category it began to be realized how errors of angle measurement might occur. The following example shows what can take place in the transfer of strain:—

In one Wild theodolite the prism holder in the side of one standard which, when turned to and fro, permits the horizontal and vertical circles to be read, was found to be improperly fitted in its box and there was a slight jamming of the prism holder as it was turned. With the prism set to view one circle the telescope axis worked freely, but with the prism set to view the other circle the telescope was very stiff on its axis—a clear case of strain in the prism box twisting the standard and communicating the strain to the cylindrical telescope axis. One can easily visualize a horizontal deflection of the telescope in such a case.

One dangerous feature of axis strain is that, unless it is excessive, it can be detected and localized only by a specially designed test, with apparatus which is not always available to a surveying organization. It may not be

noticeable by any "feel" of definite stiffness; also while appreciable stiffness may be due to strain it may be due to gummy oil on the axes. Axes known to be free from strain, but which have not been cleaned and re-oiled for several months, may "feel" stiffer than axes known to contain strain but which have just been cleaned and oiled.

Another dangerous feature of changing axis strain is that it is not detected by the ordinary standards of comparison used in field practice, such as the agreement of the various measurements of angles, and is not eliminated by the repetition of observations.

Another consideration was that if there were some strain in the telescope axis, elevation or depression of the telescope between sights on different stations could vary the amount of strain and so result in a relative movement between the collimation axis and the reference line of the horizontal circle micrometer. Further possible errors might arise from warping of the male and female alidade axis with consequent variable strains at different azimuths of the telescope.

It should be emphasized, too, that there are several possible sources of strain which may finally result in errors of horizontal angle measurement. The axis systems (alidade and telescope) together with their connections to the standards, must be considered as a unit; any strain in any part of the unit may react on any other part; for satisfactory performance the possibility of strain being introduced to any part of it must be eliminated entirely.

Detecting Axis Strain in Wild Theodolites

The first definite indication that a theodolite was not giving accurate results came from an analysis of rounds of readings, either on field lights or on collimators. It was noticed that the difference between direct and reverse readings—on horizontal sights this equals double the collimation error—was not always the same on all of the objects sighted on; in other words some of the angles measured with "telescope direct" were consistently different from those with "telescope reverse". One notable example occurred during a field test in 1932 in the measurement of an angle of about 185° with Wild theodolite No. 561, in which noticeable stiffness due to warping of the alidade axis existed.

Each of the sixteen measurements indicated the same trend as the means shown in Table VI.

TABLE VI

DIFFERENCE BETWEEN DIRECT AND REVERSE READINGS

—	Difference between direct and reverse readings		Angle between Station *A* and Station *B*	
	On station *A*	On station *B*	Telescope direct	Telescope reversed
			185° 03′	
Mean of 16 measurements	04″.84	01″.29	03″.96	07″.51

The angle shown in Table VI was the only angle in a round of four which showed trouble of this order of magnitude. This indicated that, while a systematic error of some kind existed in this instrument, it varied at different azimuths of the telescope. Variable strain in the alidade axis was suggested as the cause of this systematic error.

TABLE VII

DIFFERENCE BETWEEN DIRECT AND REVERSE READINGS (MEANS OF EIGHT RESULTS IN EACH CASE)

B	*C*	*D*	*A*	Closure
11″.72	10″.11	10″.27	12″.38	11″.78
	10″.82	09″.79	11″.75	10″.92
		11″.00	12″.00	10″.96

Another example of the same occurrence, though much smaller in amount, when sighting on collimators, is given in Table VII.

It is apparent that the results on collimator *A* are uniformly larger than on other collimators, while those on *C* and *D* are smaller.

It was realized that errors in angle measurement produced by axis strain could be detected in the laboratory only by changing the positions of the leveling screws, thus altering the relation of the alidade axis and its socket. Test angles were therefore measured between two collimators, the leveling screws being placed in three positions 120° apart in each third of the test. Table VIII shows the difference fairly plainly; the results in each case are the means of 16 rounds of readings, eight with telescope direct and eight with telescope reverse, in eight positions of the horizontal circle.

TABLE VIII

ANGLES MEASURED WITH THEODOLITE WITH STRAINED AXES

Marked footscrew at	Angle (seconds only) between collimators *A* and *B*
0°	38″.54
120°	37″.80
240°	40″.24

The three mean values should have a maximum variation of two- or three-tenths of a second (see Table XI), and it is evident that a different systematic error existed in each third of the measurements; the cause could be due only to changing strain in the alidade axis, as no other factor was altered.

A method had now been found for detecting the effect of strain, and this method could be conveniently applied to each part of the full test program outlined in Table II. Each third of each part of the program was taken with the footscrews in different positions, and the probable error of an angle was calculated in the same manner as before, treating systematic errors as accidental ones. Table IX illustrates the probable errors derived from this type of test.

TABLE IX

PROBABLE ERRORS WITH THEODOLITES WITH STRAINED AXES

Part	Wild No. 558	Wild No. 562
1	±0″.151	±0″.219
2	±0″.102	±0″.065
3	±0″.262	±0″.139
4	±0″.240	—
Mean	±0″.189	±0″.141

For comparison, the results with Wild No. 558 without interchanging

the position of the footscrews, as shown in Table V, may be consulted. These mean probable errors are obviously larger than the standard of comparison ($\pm 0''.1$), and these theodolites, among others, were judged to have strained axes.

All of the evidence, of which the above are only examples, indicated that angular errors of appreciable magnitude—of the order of 2″ to 4″—may have resulted from strain or other axis trouble in at least nine of the 10 Wild theodolites examined. Attention was then devoted to its elimination.

New Axis Design

As stated previously, geometrical precision is not possible in practice, and even when closely approached its permanence is questionable. Attention was hence devoted to securing a form of axis system in which the unavoidable inexactitude in manufacture or secular change would have no effect on the instrument indications. A well known method is to apply the principle of kinematical design (1). In the new form every endeavor was made to introduce as few changes as possible to the theodolites. For a new instrument it would be possible to improve and extend the changes here described. The changes indicated below were made in the shops of the National Research Council. About four days were required by one instrument maker to complete the mechanical work on each theodolite.

Alidade Axis

Figs. 2*a* and 2*b* show the original and modified alidade axes respectively.

A three-point ball bearing (1 in Fig. 2*b*)—three balls only were used in the first cases, but as it was feared that damage might occur from vibration during transit, nine balls were fitted later—replaces the ball thrust bearing (2 in Fig. 2*a*) and plain cylindrical locating bearing (3 in Fig. 2*a*). At the lower end of the axis (4 in Fig. 2*b*) two-thirds of the length of the bearing was relieved, so that there would be negligible constraint with a slightly warped axis. This end of the bearing is not subject to much load and needs to have only sufficient surface to resist ordinary wear. A better design might be two solid pads placed 120° apart with a third spring supported pad. This design could not be employed in this instrument without large changes of design.

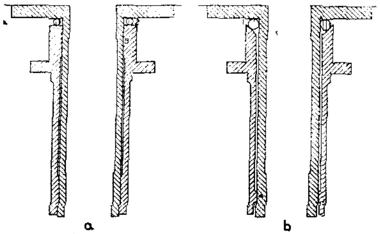

FIG. 2. *Original and modified alidade axis.*

There was strong evidence that the lower part of the original axis was too tight in some cases as, even with the remodeled axis, the tolerance of the lower bearing surface had

to be increased before improved results were obtained. It was found to be safer to have the lower bearing slightly loose than the least bit tight.

Telescope Axis

As indicated in Fig. 3, at each end of the telescope axis two-thirds of the length of the bearing cylinder was relieved to reduce the likelihood of strain being communicated through these bearings to the collimation axis, and a circumferential section of 60° was relieved entirely at the top of the bearing. By this latter device there was avoided the fault called "roll and slip" which had been found in several of the theodolites. This is the slight tendency of the telescope axis to climb in its bearing as the telescope is revolved. In the exaggerated case shown in Fig. 4, where the outer cylinder revolves on the inner one, the first action is a rolling motion, and the point of contact moves from M to M'. As the motion continues the position of M' changes and may return to M. It is quite easy to realize that, owing to the lack of exact symmetry already mentioned, the roll and slip may not be the same at both ends of the telescope axis and slight deflections of the telescope result. This fault was definitely detected during some of the tests and showed itself by irregularities in readings shortly after the telescope was transited.

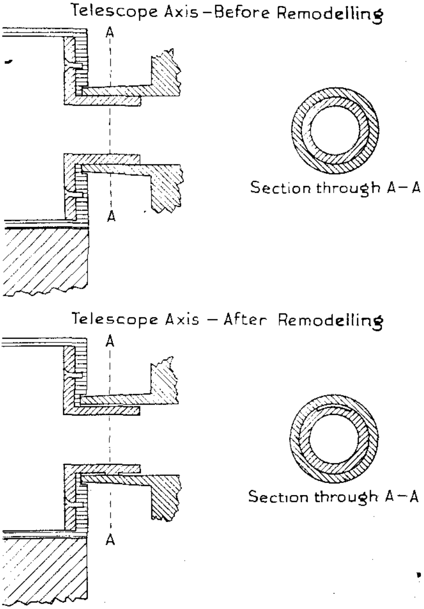

FIG. 3. *Original and modified telescope axis.*

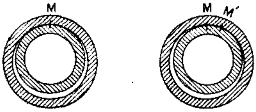

FIG. 4. *Roll and slip of telescope axis.*

One other small source of trouble was traced to the telescope axis. The small horizontal spring which actuates a horizontal plunger through which the electric current is transferred from standards to telescope was found to be too strong and this accentuated the climb of the axis.

It is needless to add that workmanship of the highest grade is necessary in fitting the new axes, but it is felt that, while no amount of skill can preclude the possibility of strain errors with the older type, with reasonable care in the shop, troubles of this nature should not occur with the new form. It may be added that the new type of axis should be cheaper to construct than the older form.

In assembling the remodeled telescope axis the following procedure is largely independent of "feel". The block containing the micrometer at the top of one standard has two fixed dowels; this block is screwed tight. Any adjustment is made with the block on top of the other standard, the screw dowel of which is removed. The free block is then moved sideways until the telescope tightens in the fixed block; it is then moved to the other side until the telescope again tightens on the other side of the fixed block. The mean of these two positions of the free block is used as the proper position. Straight edges are then placed along the outer edges of the two blocks and the free block is twisted until the straight edges are parallel. A small amount of end play in the telescope is advisable. The block is then screwed tight, the screw dowel being left out.

If it is found that the two ends of telescope axis are not truly in line or if the cylindrical bearing surfaces are not square with the outer faces of the blocks preliminary adjustments are required.

No further trouble has been experienced with this modified telescope axis.

Tests with Modified Wild Theodolites

Following the remodeling each instrument was subjected to the full test program described above employing the same observers. Table X exemplifies the probable errors derived from the remodeled theodolites.

TABLE X

PROBABLE ERRORS DERIVED FROM THE REMODELED THEODOLITES

Part	Wild No. 554	Wild No. 558	Wild No. 559	Wild No. 560	Wild No. 561
1	±0″.080	±0″.089	±0″.117	±0″.100	±0″.073
2	±0″.038	±0″.068	±0″.042	±0″.041	±0″.060
3	±0″.084	±0″.099	±0″.085	±0″.105	±0″.032
4	±0″.109	±0″.070	±0″.055	±0″.076	±0″.105
Mean	±0″.079	±0″.081	±0″.075	±0″.080	±0″.068

These mean probable errors are smaller than the standard set for comparison purposes (±0″.1) and are still smaller than those obtained with

similar theodolites which exhibited strained axes. It has therefore been concluded that systematic errors have been eliminated from these theodolites and that accidental errors have been slightly reduced.

TABLE XI

ANGLES MEASURED WITH THEODOLITE WITH REMODELED AXES

Marked footscrew at	Angle (seconds only) between collimators *A* and *B*
0°	51″.68
120°	51″.65
240°	51″.64

It is interesting to note how the theodolite with remodeled axes acts compared to its action with strained axes. An angle was measured between two collimators with the footscrews moved between sets with the following results. Each of the results in Table XI is the mean of 16 rounds of readings, eight with telescope direct and eight reverse, in eight positions of the horizontal circle.

TABLE XII

DIFFERENCE BETWEEN DIRECT AND REVERSE READINGS (MEANS OF EIGHT RESULTS IN EACH CASE)

D	*A*	*B*	*C*	Closure
14″.38	14″.08	14″.21	14″.13	14″.36
	14″.25	14″.05	14″.24	14″.13
		14″.13	14″.18	14″.19

Again, consider the differences between direct and reverse readings on different collimators with a theodolite with remodeled axes (Table XII).

The results in Tables XI and XII are strikingly better than those shown in Tables IX and VIII and indicate that axis trouble has been largely eliminated.

Observational Procedure to Reduce Effect of Strain and Roll

There will be operators of Wild theodolites who cannot conveniently test their instruments to determine whether they have strained axes, or who cannot conveniently have the changes made which are suggested in this paper, but who wish to obtain the best results of which their instrument is capable. The following precautions and observation procedure are suggested by this investigation.

1. The footscrews must be adjusted tighter than is necessary with heavier theodolites. Even with the footscrews tight, however, there may be unsteadiness of the theodolite owing to the casing of the footscrew assembly (*a*, Fig. 5), which screws into the base of the theodolite, being imperfectly seated at the contact shown by the arrows in Fig. 5. Where this is the case a marked improvement in the steadiness of the theodolite is obtained by unscrewing the assembly from the base, after removing the grub screw, and scraping the base to give a perfect seating.

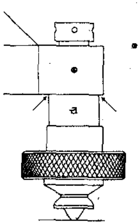

FIG. 5. *Imperfectly seated casing of footscrew assembly is a possible cause of instability of theodolite.*

2. If there is any suggestion of stiffness in the action of the alidade axis it should be lapped to a loose fit. "Wobble" of the axis, contrary to most beliefs, is not a serious fault, and a slack centre is preferable to a tight one.

3. The most meticulous care should be taken to avoid any stiffness in the telescope action at any point. First the blocks at the tops of the standards should be manip-

ulated to obtain the utmost freedom of action. If stiffness cannot be eliminated in this manner it may be necessary to dismantle the vertical circle case (six screws hold the two halves together) and other parts of the axis, carefully work the plane surfaces and exercise great care in replacing the screws to obtain even tension.

4. The tests have demonstrated that axis strain acts differently as the telescope is swung in azimuth. To minimize its effect the leveling screws should be changed in position as frequently as possible. For instance, any program might be divided into three parts and the leveling screws moved 120° between parts.

5. Whenever the telescope is moved the top centre of the telescope might be tapped to avoid error due to roll and slip.

Appendix

Other Probable Errors

In the test program described above the circle is closed in the middle of each position, *i.e.*, the telescope is pointed on the initial in the middle of each position before and after reversing the telescope. The error of closure provides the data for calculating the probable error of a measurement of an angle due to combined telescope pointing and micrometer reading errors.

Similarly for all other directions the differences between the individual position results and the mean of a set give the data for calculating the probable error of an angle due to errors of combined telescope pointing, micrometer reading and graduation.

The effect of graduation errors can easily be separated from that of telescope pointing and micrometer reading.

Telescope, Micrometer and Graduation Errors

The probable errors of measurement due to the above errors were calculated from a full test program with one Wild theodolite and with two older-type theodolites which had given years of satisfactory service.

TABLE XIII

PROBABLE ERRORS OF ANGLE MEASUREMENT DUE TO POINTING, READING AND GRADUATION ERRORS

Probable error of measurement of angle (r_{16}) due to telescope pointing and micrometer reading errors		Probable error of measurement of angle (r_{16}) due to graduation errors	
Three-micrometer Cooke theodolite	±0″.07	Three-micrometer Cooke theodolite	±0″.13
Two-micrometer Parkhurst theodolite	±0″.09	Two-micrometer Parkhurst theodolite	±0″.16
Wild theodolite No. 558	±0″.08	Wild theodolite No. 558	±0″.13

The data in Table XIII corroborate the conclusions arrived at from other tests, that no trouble from these sources need be anticipated in this

particular theodolite of the Wild type. Similar data exist for a number of other Wild theodolites.

Acknowledgments

The main share of the observations on which this investigation depended were carried on by two officers of the Geodetic Survey of Canada—G. F. Dalton and E. M. Medlen. Invaluable advice and consultation were received from R. H. Field of the Department of Physics and Engineering of the National Research Council. The machine work involved in remodeling the axes of the Wild theodolite was done by A. A. Barks of the National Research Council staff.

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A FIELD TEST OF PRIMARY TRIANGULATION THEODOLITES[1]

BY J. L. RANNIE[2]

Abstract

A form of test of the accuracy of precision theodolites is described in which angles were measured out of doors at night with different instruments, the targets being small lights placed at about two miles from the observing station. Probable errors computed from the observations were the basis of comparison and it is shown how these were derived and employed. This test was complementary to a laboratory test on similar instruments.

Introduction

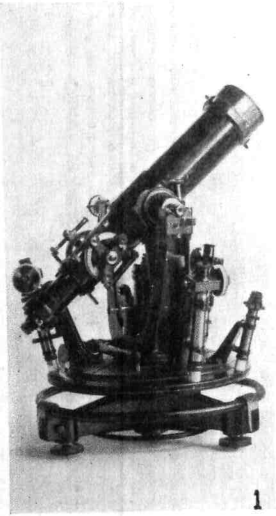
During May, June and July, 1933, an investigation was carried out at Geodetic Survey triangulation station "Ottawa", under conditions approximating those met in field work, to compare the respective capabilities of three triangulation theodolites of different types for correctly measuring horizontal angles. The test was undertaken as a confirmation, under different observational and atmospheric conditions, of conclusions arrived at in the laboratory (3).

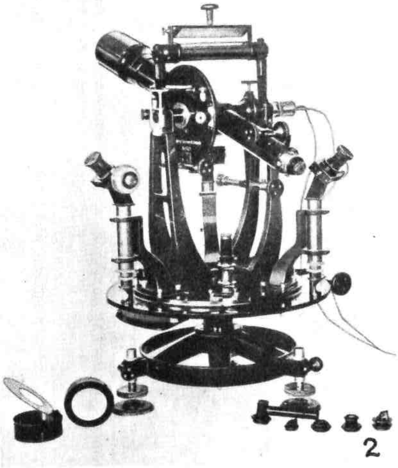
The basis of comparison of different theodolites both in laboratory and field tests was the relative size of the probable error of measurement of an angle, a lower probable error indicating a higher precision.

In the laboratory tests (3) angles were measured on collimators, and unexpected difficulties due to minute errors of focus of the collimators and theodolites and to tiny differences of centering of different theodolites, made it necessary to confine the laboratory investigation to independent tests of each theodolite. While the laboratory test was therefore suitable for the detection of accidental errors and also for such systematic errors as were known to exist, it might not disclose unknown systematic errors. In the field test, errors due to the above causes were negligible so that, in addition to the conclusions based on the performance of independent theodolites, it was possible to base conclusions on a direct comparison of angles measured with two theodolites, and hence disclose any unknown systematic errors. This constituted an important advantage of the field test over the laboratory investigations, and made it valuable as a final confirmation of results reached in the laboratory.

The disadvantages of any field test compared to laboratory tests are several. Delays are occasioned in the field by bad weather; a much larger number of observations is needed in the field than in the laboratory, owing to poorer targets and changing atmospheric conditions, to produce results which may be relied on; owing to the greater uniformity of laboratory conditions any abnormal deviations can be much more easily recognized and traced to their source. For these reasons the bulk of the testing of the theodolites took place in the laboratory, the field test being of a confirmatory nature only.

[1] *Manuscript received January 18, 1934.*
Contribution from the Geodetic Survey of Canada, Department of the Interior, Ottawa.
[2] *Chief of the Triangulation Division, Geodetic Survey of Canada, Department of the Interior, Ottawa.*





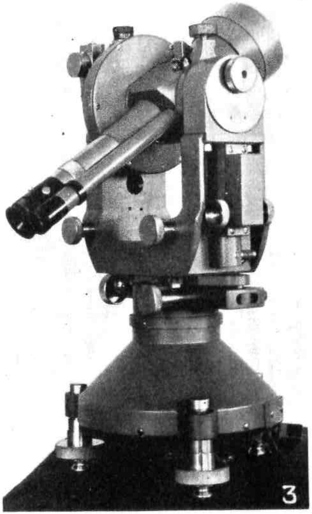


FIG. 1. *Cooke 12-in. three-micrometer theodolite.* FIG. 2. *Kern 12-in. three-micrometer theodolite. For this investigation this instrument was rearranged to make it a two-micrometer instrument.* FIG. 3. *Wild precision theodolite, $5\frac{1}{2}$-in. horizontal circle.*

Theodolites Compared

Theodolites of three types were compared. One theodolite was a heavy Cooke 12-in., three-micrometer microscope instrument; the second was a lighter 12-in., three-micrometer Kern theodolite which was changed before the test by removing one micrometer and re-arranging the other two to make it a two-micrometer theodolite; the third was a Wild precision theodolite with a 5½-in. horizontal circle. The first two theodolites had given satisfactory field performance over a lengthy period. They had more or less conventional steel conical alidade axes with the horizontal or telescope axis lying in open *Y*'s; the Wild had cylindrical alidade and telescope axes, and was one which had been shown in the laboratory to be largely free from axis strain and other sources of error. The instruments are illustrated in Figs. 1, 2 and 3.

Layout for Field Test

For the purpose of the test four stations were selected about two miles distant from triangulation station "Ottawa" of the Geodetic Survey of Canada. Fig. 4 shows the disposition of the stations.

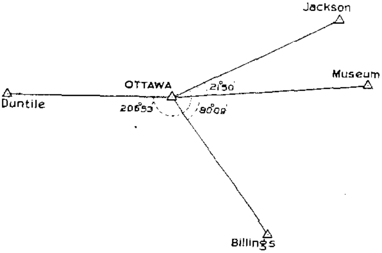


FIG. 4. *Arrangement of stations employed in field test.*

The observing station, Ottawa, was situated about 60 ft. above the ground level on the concrete roof of the stair shaft of the Dominion Observatory. The instrument stand was a wooden tripod cemented to the roof, and the observer stood on a platform separated from the tripod and supported only on the stone walls.

Stations Jackson and Museum were on the roofs of buildings, and the lines to them passed over the city of Ottawa, while Billings and Duntile were ground stations, and the lines to them passed mainly over open country.

All the lines were from 50 to 100 ft. above the buildings or ground along the line for a greater part of the distance.

The targets were small electric automobile headlamps with parabolic reflectors of the type used in Canada on primary triangulation. Current was supplied from the regular city lighting circuit by using small bell-ringing transformers, and the intensity of the lights was regulated by rheostats. The faces of the lamps were covered, except for a centralized vertical slit less than ⅛ in. in width; thus only light from the top and bottom of the reflector was seen from the instrument station. When seen in the telescope under favorable atmospheric conditions the light appeared as two fine dots in a vertical plane, an ideal light for making accurate pointings between the

two vertical wires of the theodolite. Atmospheric disturbances usually tended to fuse and enlarge the dots and made them jumpy, an ordinary field condition, in which case the precision of the telescope pointings was noticeably lowered.

In the telescopes of the Cooke and Kern theodolites the diaphragms were fitted with two vertical spider webs about 30 sec. of arc apart. For pointings, images were placed midway between the two wires. The telescope of the Wild theodolite was fitted with a glass diaphragm having two short, nearly vertical converging lines ruled thereon; at one end the lines were 20 sec. apart and at the other 40 sec.; images of different sizes were placed at such heights in the field of view that there were equal side spaces on different lights between the lines and the image.

Program of Test

The three theodolites were tested in pairs, and the program with each pair was so arranged that the probable error of the measurement of an angle could be calculated in two ways:

1. For each theodolite of the pair, considering only the angular measurements with that instrument.
2. For each theodolite of the pair in terms of the mean result with that pair.

It was anticipated that the first method would give a comparison of the accidental errors of measurement with different theodolites. The second method would in addition indicate whether any systematic errors of measurement existed in any of the theodolites.

The test consisted of nine parts for each pair, each part being independent of the others and being designed to be completed in about three to four hours on one night. The test therefore was completed in 27 nights.

The program comprised the measurement of single and overlapping angles, from an adjustment of which the most probable values of the angles and the above probable errors were derived.

In Table I is given the program of Part 1 for one pair of theodolites.

TABLE I

PROGRAM OF TEST

Theodolite	Position	Circle setting on initial	Points sighted on	Observer
Cooke	I	0°-02′-00″ 0°-02′-00″ 0°-02′-00″	*J M B D J* *M B D M* *B D B*	*A*
Kern	I	0°-02′-00″ 0°-02′-00″ 0°-02′-00″	*J M B D J* *M B D M* *B D B*	*B*
Kern	II	90°-03′-00″ 90°-03′-00″ 90°-03′-00″	*B D B* *M B D M* *J M B D J*	*A*
Cooke	II	30°-03′-00″ 30°-03′-00″ 30°-03′-00″	*B D B* *M B D M* *J M B D J*	*B*

The other eight parts differed from the above only in the circle readings on the initial and in the station chosen as the first initial. These differences are indicated in Table II.

TABLE II

CIRCLE POSITIONS WITH 3– AND 2–MICROMETER THEODOLITES

Part	Position	Cooke theodolite	Kern and Wild theodolites	First initial	Part	Position	Cooke theodolite	Kern and Wild theodolites	First initial
1	I	0°–02′–00″	0°–02′–00″	Jackson	6	XI	23°–02′–00″	50°–02′–00″	Museum
	II	30°–03′–00″	90°–03′–00″			XII	53°–03′–00″	140°–03′–00″	
2	III	10°–02′–00″	10°–02′–00″	Jackson	7	XIII	6°–02′–00″	60°–02′–00″	Billings
	IV	40°–03′–00″	100°–03′–00″			XIV	36°–03′–00″	150°–03′–00″	
3	V	20°–02′–00″	20°–02′–00″	Jackson	8	XV	16°–02′–00″	70°–02′–00″	Billings
	VI	50°–03′–00″	110°–03′–00″			XVI	46°–03′–00″	160°–03′–00″	
4	VII	3°–02′–00″	30°–02′–00″	Museum	9	XVII	26°–02′–00″	80°–02′–00″	Billings
	VIII	33°–03′–00″	120°–03′–00″			XVIII	56°–03′–00″	170°–03′–00″	
5	IX	13°–02′–00″	40°–02′–00″	Museum					
	X	43°–03′–00″	130°–03′–00″						

In the above program the following points are to be noted:—

1. In the column "Points sighted on" the letters *J*, *M*, *B* and *D* represent stations Jackson, Museum, Billings and Duntile, respectively (Fig. 4).

2. Each set of readings, called a position, consisted of two rounds, one clockwise with telescope "direct" followed by a counterclockwise round with telescope "reverse". The mean of the two constituted a position.

3. The circle settings were distributed equally around the circle.

4. The minutes and seconds of the circle settings were selected to eliminate, as far as possible, any error of "run".

5. The order of making the measurements was designed to eliminate the effect of any regular change in the apparent position of a light due to atmospheric conditions.

6. Frequently a second observer helped in reading micrometers, and took a turn in pointing the telescope. Where this occurred care was taken to have each observer make the pointings as shown in the column headed "Observer".

7. On successive nights the footscrews were changed in position by 120°, so that any differential axis strain might be detected.

8. To eliminate the effect of any variable personal equation of pointing (2) a reversing prism was attached to the eyepieces of the telescopes, and the prism was set to view the image "direct" in the one half of each position and "reversed horizontally" in the other half.

9. To check the action of the theodolite, return readings were made on the initial station in the middle of each set. The mean value of the readings on the initial was used in calculating the angles.

A sample of the reduced readings taken in one part of the above program, rearranged for ease of computation, is shown in Table III.

TABLE III

REDUCED READINGS OF MEASUREMENTS ON ONE NIGHT

Cooke theodolite					*Kern theodolite*					Sequence of readings	
Jackson	Museum	Billings	Duntile	Closure	Jackson	Museum	Billings	Duntile	Closure	Cooke	Kern
0° 00′ 00″.0	21° 49′ 34″.3 33″.8	80° 08′ 33″.8 34″.3	206° 52′ 44″.1 44″.3	359° 59′ 59″.7 59″.7	0° 00′ 00″.0	21° 49′ 34″.6 34″.8	80° 08′ 34″.7 34″.9	206° 52′ 44″.7 43″.2	359° 59′ 59″.4 60″.5	1 12	4 9
	*34″.05 †34″.20	34″.05 34″.20	44″.20 44″.35	59″.70		34″.70 34″.72	34″.80 34″.82	43″.95 43″.97	59″.95		
	0° 00′ 00″.0	58° 18′ 59″.2 60″.9	185° 03′ 10″.0 09″.2	359° 59′ 60″.0 59″.9		0° 00′ 00″.0	58° 18′ 60″.9 62″.1	185° 03′ 10″.3 09″.2	359° 59′ 60″.0 59″.4	2 11	5 8
		*60″.05 †60″.07	09″.60 09″.62	59″.95			61″.50 61″.65	09″.75 09″.90	59″.70		
		0° 00′ 00″.0	126° 44′ 09″.0 09″.0	359° 59′ 60″.3 59″.7			0° 00′ 00″.0	126° 44′ 09″.4 09″.7	359° 59′ 59″.9 59″.7	3 10	6 7
			*09″.00 †09″.00	60″.00				09″.55 09″.65	59″.80		

* *Observed mean.* † *Mean corrected for closure.*

Adjustment of Observations

The principle (1) of station adjustment was used to reduce the observations. In Fig. 5 the measured angles are: x, y and z; $y-x$ and $z-x$; and $z-y$. From these measured values six observation equations connecting the three independent unknowns x, y and z can be formed, the solution of which by least squares gives the most probable (adjusted) values of the angles. The differences between the adjusted and measured values give six residuals from which the probable error of the measurement of the angle from two positions can be derived by means of the usual formula

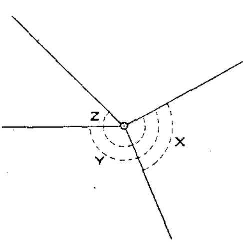


FIG. 5. *Independent angles measured.*

$$r_1 = \pm 0.6745\sqrt{\frac{\Sigma v^2}{n-q}}.$$

The results for each night's observations with each theodolite are adjusted separately by the above method, and a comparison of the probable errors

The other eight parts differed from the above only in the circle readings on the initial and in the station chosen as the first initial. These differences are indicated in Table II.

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CIRCLE POSITIONS WITH 3- AND 2-MICROMETER THEODOLITES

Part	Position	Cooke theodolite	Kern and Wild theodolites	First initial	Part	Position	Cooke theodolite	Kern and Wild theodolites	First initial
1	I	0°-02′-00″	0°-02′-00″	Jackson	6	XI	23°-02′-00″	50°-02′-00″	Museum
	II	30°-03′-00″	90°-03′-00″			XII	53°-03′-00″	140°-03′-00″	
2	III	10°-02′-00″	10°-02′-00″	Jackson	7	XIII	6°-02′-00″	60°-02′-00″	Billings
	IV	40°-03′-00″	100°-03′-00″			XIV	36°-03′-00″	150°-03′-00″	
3	V	20°-02′-00″	20°-02′-00″	Jackson	8	XV	16°-02′-00″	70°-02′-00″	Billings
	VI	50°-03′-00″	110°-03′-00″			XVI	46°-03′-00″	160°-03′-00″	
4	VII	3°-02′-00″	30°-02′-00″	Museum	9	XVII	26°-02′-00″	80°-02′-00″	Billings
	VIII	33°-03′-00″	120°-03′-00″			XVIII	56°-03′-00″	170°-03′-00″	
5	IX	13°-02′-00″	40°-02′-00″	Museum					
	X	43°-03′-00″	130°-03′-00″						

In the above program the following points are to be noted:—

1. In the column "Points sighted on" the letters *J*, *M*, *B* and *D* represent stations Jackson, Museum, Billings and Duntile, respectively (Fig. 4).

2. Each set of readings, called a position, consisted of two rounds, one clockwise with telescope "direct" followed by a counterclockwise round with telescope "reverse". The mean of the two constituted a position.

3. The circle settings were distributed equally around the circle.

4. The minutes and seconds of the circle settings were selected to eliminate, as far as possible, any error of "run".

5. The order of making the measurements was designed to eliminate the effect of any regular change in the apparent position of a light due to atmospheric conditions.

6. Frequently a second observer helped in reading micrometers, and took a turn in pointing the telescope. Where this occurred care was taken to have each observer make the pointings as shown in the column headed "Observer".

7. On successive nights the footscrews were changed in position by 120°, so that any differential axis strain might be detected.

8. To eliminate the effect of any variable personal equation of pointing (2) a reversing prism was attached to the eyepieces of the telescopes, and the prism was set to view the image "direct" in the one half of each position and "reversed horizontally" in the other half.

9. To check the action of the theodolite, return readings were made on the initial station in the middle of each set. The mean value of the readings on the initial was used in calculating the angles.

A sample of the reduced readings taken in one part of the above program, rearranged for ease of computation, is shown in Table III.

TABLE III

REDUCED READINGS OF MEASUREMENTS ON ONE NIGHT

Cooke theodolite					Kern theodolite					Sequence of readings	
Jackson	Museum	Billings	Duntile	Closure	Jackson	Museum	Billings	Duntile	Closure	Cooke	Kern
0° 00′	21° 49′	80° 08′	206° 52′	359° 59′	0° 00′	21° 49′	80° 08′	206° 32′	359° 59′		
00″.0	34″.3	33″.8	44″.1	59″.7	00″.0	34″.6	34″.7	44″.7	59″.4	1	4
	33″.8	34″.3	44″.3	59″.7		34″.8	34″.9	43″.2	60″.5	12	9
	*34″.05	34″.05	44″.20	59″.70		34″.70	34″.80	43″.95	59″.95		
	†34″.20	34″.20	44″.35			34″.72	34″.82	43″.97			
	0° 00′	58° 18′	185° 03′	359° 59′		0° 00′	58° 18′	185° 03′	359° 59′		
	00″.0	59″.2	10″.0	60″.0		00″.0	60″.9	10″.3	60″.0	2	5
		60″.9	09″.2	59″.9			62″.1	09″.2	59″.4	11	8
		*60″.05	09″.60	59″.95			61″.50	09″.75	59″.70		
		†60″.07	09″.62				61″.65	09″.90			
		0° 00′	126° 44′	359° 59′			0° 00′	126° 44′	359° 59′		
		00″.0	09″.0	60″.3			00″.0	09″.4	59″.9	3	6
			09″.0	59″.7				09″.7	59″.7	10	7
			*09″.00	60″.00				09″.55	59″.80		
			†09″.00					09″.65			

* *Observed mean.* ? *Mean corrected for closure.*

Adjustment of Observations

The principle (1) of station adjustment was used to reduce the observations.

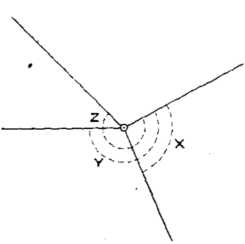

FIG. 5. *Independent angles measured.*

In Fig. 5 the measured angles are: x, y and z; $y-x$ and $z-x$; and $z-y$. From these measured values six observation equations connecting the three independent unknowns x, y and z can be formed, the solution of which by least squares gives the most probable (adjusted) values of the angles. The differences between the adjusted and measured values give six residuals from which the probable error of the measurement of the angle from two positions can be derived by means of the usual formula

$$r_2 = \pm 0.6745\sqrt{\frac{\Sigma v^2}{n-q}}.$$

The results for each night's observations with each theodolite are adjusted separately by the above method, and a comparison of the probable errors

gives the first basis for comparing the precision of measurements with each theodolite, as far as accidental errors are concerned.

The second basis of comparison is predicated on the *a priori* assumption that both instruments of a pair are capable of angular measurements of the same order of precision. This assumption was later found slightly erroneous. Hence it is assumed that the arithmetic mean or mean adjusted value of the measurements with two theodolites is the most probable value for that night. The differences between the measured and mean adjusted values give residuals from which probable errors are obtained for each theodolite by the employment of the above formula. The mean adjusted value of the angles is obtained by combining in one adjustment the six observation equations for each theodolite, together with three condition equations which require that the adjusted values of x, y and z from the two theodolites are equal.

The adjustment of the set in Table III and the calculation of the probable errors is given below in skeleton.

The observation equations are of the form

$$v = X_a - X_m + \Delta x \ ,$$

where X_a is the assumed value of any angle, X_m the measured value of the same angle, and Δx the most probable correction to the assumed value.

The condition equations which equalize the adjusted values for each theodolite are of the form

$$X_a + \Delta x = X_a + \Delta x'$$

or

$$\Delta x = \Delta x'$$

These condition equations are applied to the normal equations.

The numerical work of reducing the set in Table III is given in Table IV.

TABLE IV

Calculation of absolute terms

Symbol	Measured angles (seconds only)		Assumed value	Assumed − measured	
	Cooke	Kern		Cooke	Kern
x	34″.20	34″.72	34″.20	0″	−0″.52
y	34″.20	34″.82	34″.20	0″	−0″.62
z	44″.35	43″.97	44″.35	0″	+0″.38
$y-x$	60″.07	61″.65	60″.00	−0″.07	−1″.65
$z-x$	09″.62	09″.90	10″.15	+0″.53	+0″.25
$z-y$	09″.00	09″.65	10″.15	+1″.15	+0″.50

The observation equations and the condition equations are shown in tabular form in Table V.

TABLE V

SET-UP OF OBSERVATION AND CONDITION EQUATIONS

Cooke			Kern			
Δx	Δy	Δz	$\Delta x'$	$\Delta y'$	$\Delta z'$	l
Observation equations						
+1						$0 = v_1$
	+1					$0 = v_2$
		+1				$0 = v_3$
−1	+1					$-0.07 = v_4$
−1		+1				$+0.53 = v_5$
	−1	+1				$+1.15 = v_6$
			+1			$-0.52 = v_7$
				+1		$-0.62 = v_8$
					+1	$+0.38 = v_9$
			−1	+1		$-1.65 = v_{10}$
			−1		+1	$+0.25 = v_{11}$
				−1	+1	$+0.50 = v_{12}$
Condition equations						
+1			−1			$+0 = 0$
	+1			−1		$+0 = 0$
		+1			−1	$+0 = 0$

The resulting normal equations to effect the desired corrections to the assumed value of the angles are shown in Table VI. K_1, K_2, and K_3 are undetermined multipliers.

TABLE VI

NORMAL EQUATIONS

Δx	Δy	Δz	$\Delta x'$	$\Delta y'$	$\Delta z'$	K_1	K_2	K_3	
+3	−1	−1				+1			−0.46
	+3	−1					+1		−1.22
		+3						+1	+1.68
			+3	−1	−1	−1			+0.88
				+3	−1		−1		−2.77
					+3			−1	+1.33
						0			0
							0		0
								0	0

The first basis of comparison is obtained by solving the normal equations exclusive of the three condition equation columns at the right. By this process the corrections to the assumed values are obtained for each theodolite separately:—

$$\Delta x = +0''.115 \qquad \Delta x' = -0''.030$$
$$\Delta y = +0''.305 \qquad \Delta y' = +0''.883$$
$$\Delta z = -0''.420 \qquad \Delta z' = -0''.092$$

from which are obtained

$$\Delta y - \Delta x = +0''.190 \qquad \Delta y' - \Delta x' = +0''.913$$
$$\Delta z - \Delta x = -0''.535 \qquad \Delta z' - \Delta x' = -0''.062$$
$$\Delta z - \Delta y = -0''.725 \qquad \Delta z' - \Delta y' = -0''.975$$

With these data Table VII may be prepared.

TABLE VII

CALCULATIONS OF RESIDUALS FOR FIRST BASIS OF COMPARISON

Symbol	Assumed value	Adjusted values		Measured values		Adjusted − measured	
		Cooke	Kern	Cooke	Kern	Cooke	Kern
x	34″.20	34″.31	34″.17	34″.20	34″.72	+0″.11	−0″.55
y	34″.20	34″.50	35″.08	34″.20	34″.82	+0″.30	+0″.26
z	44″.35	43″.93	44″.26	44″.35	43″.97	−0″.42	+0″.29
$y-x$	60″.00	60″.19	60″.91	60″.07	61″.65	+0″.12	−0″.74
$z-x$	10″.15	09″.62	10″.09	09″.62	09″.90	0″	+0″.19
$z-y$	10″.15	09″.43	09″.18	09″.00	09″.65	+0″.43	−0″.47
						$\Sigma v^2 = 0''.4778$	1″.2581

$$r_2\ (\text{Cooke}) = \pm 0.6745\sqrt{\frac{0.4778}{6-3}} = \pm 0''.269$$
$$r_2\ (\text{Kern}) = \pm 0''.437$$

The second basis of comparison is obtained by solving the normal equations inclusive of the three condition equation columns. By this method the following values of the corrections to the assumed values are found:—

$$K_1 = +0''.669\ ,\quad \Delta x = +0''.043 = \Delta x'$$
$$K_2 = -0''.775\ ,\quad \Delta y = +0''.594 = \Delta y'$$
$$K_3 = -0''.275\ ,\quad \Delta z = -0''.256 = \Delta z'$$

from which are obtained

$$\Delta y - \Delta x = +0''.551 = \Delta y' - \Delta x'$$
$$\Delta z - \Delta x = -0''.299 = \Delta z' - \Delta x'$$
$$\Delta z - \Delta y = -0''.850 = \Delta z' - \Delta y'$$

Table VIII may now be prepared.

TABLE VIII

CALCULATION OF RESIDUALS FOR SECOND BASIS OF COMPARISON

Symbol	Assumed values	Adjusted values	Measured values		Adjusted − measured	
			Cooke	Kern	Cooke	Kern
x	34″.20	34″.24	34″.20	34″.72	+0″.04	−0″.48
y	34″.20	34″.79	34″.20	34″.82	+0″.59	−0″.03
z	44″.35	44″.09	44″.35	43″.97	−0″.26	+0″.12
$y-x$	60″.00	60″.55	60″.07	61″.65	+0″.48	−1″.10
$z-x$	10″.15	09″.85	09″.62	09″.90	+0″.23	−0″.05
$z-y$	10″.15	09″.30	09″.00	09″.65	+0″.30	−0″.35
					$\Sigma v^2 = 0''.7906$	1″.5807

NOTE:—*It will be seen that the combined adjusted values above are the means of the corresponding adjusted values obtained by the separate adjustments in Table VII.*

$$r_2\ (\text{Cooke}) = \pm 0.6745\sqrt{\frac{0.7906}{6-3}} = \pm 0''.346$$
$$r_2\ (\text{Kern}) = \pm 0''.490$$

Results of Test

The nine parts with each of the three pairs of theodolites were adjusted as shown in the above example, and the probable errors (r_2) were as shown in Table IX.

TABLE IX

PROBABLE ERRORS (r_2) OF MEASUREMENT OF AN ANGLE (RESULTS IN ALL CASES ARE THE MEANS OF NINE PARTS)

Theodolite combination	Adjusted separately		Adjusted together	
Cooke-Kern	*Cooke* ±0″.31	*Kern* ±0″.42	*Cooke* ±0″.45	*Kern* ±0″.53
Cooke-Wild	*Cooke* ±0″.27	*Wild no. 551* ±0″.33	*Cooke* ±0″.38	*Wild no. 551* ±0″.41
Kern-Wild	*Kern* ±0″.35	*Wild no. 551* ±0″.27	*Kern* ±0″.50	*Wild no. 551* ±0″.43

NOTE:—*From the range of the nine probable errors of which each of the above is the mean, it is judged that the above probable errors may not be correct to within 0″.03.*

Conclusions from Field Test

(i) The probable errors derived from the adjustment of the measurements of each instrument separately indicate that the Cooke theodolite and Wild theodolite No. 551 measured angles equally precisely so far as accidental errors were concerned, while the measurements with the Kern theodolite were slightly less precise. The mean probable errors for the three instruments are:—

$$\text{Cooke theodolite} = \frac{0.31+0.27}{2} = \pm 0''.29$$

$$\text{Kern theodolite} = \frac{0.42+0.35}{2} = \pm 0''.39$$

$$\text{Wild theodolite No. 551} = \frac{0.33+0.27}{2} = \pm 0''.30$$

(ii) The mean probable errors for the three instruments when the measurements were adjusted in pairs are:—

$$\text{Cooke theodolite} = \frac{0.45+0.38}{2} = \pm 0''.42$$

$$\text{Kern theodolite} = \frac{0.53+0.50}{2} = \pm 0''.51$$

$$\text{Wild theodolite No. 551} = \frac{0.41+0.43}{2} = \pm 0''.42$$

The close relative agreement of these figures with those in (i) above shows that, while the first group did not reveal the full extent of the accidental errors, no systematic errors existed in any of the three theodolites which were not revealed by the first comparison.

(iii) The above conclusions substantiate those derived from laboratory tests with respect to Wild theodolite No. 551 and give considerable confidence that no systematic errors existed in this instrument which were not disclosed in the laboratory.

(iv) From a number of other considerations it seems likely that the slight inferiority of results with the Kern theodolite is due to graduation errors which naturally disclose themselves in such a test as the above where each part of the test is based on the mean of readings in only two positions of the circle. In a regular field program, where a much larger number of circle positions is employed, the effect of such graduation errors is practically eliminated. It is also to be noted that this instrument was originally provided with three micrometers, in which condition its field performance was quite satisfactory.

Acknowledgments

A number of officers of the Geodetic Survey of Canada, Department of the Interior, participated in the series of observations which constituted the basis of this paper; others, by their discussions and suggestions contributed materially to the methods employed. Particular mention should be made of the late A. M. Grant, who began the whole investigation into the Wild theodolite and who carried on until his death, which occurred before the completion of the investigation.

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IN SITU CULTURES OF DERMATOPHYTES[1]

BY A. M. DAVIDSON[2] AND P. H. GREGORY[3]

Abstract

Studies were undertaken to correlate structure with life cycle in four common species of fungi causing skin disease in man. Hairs naturally infected by *Microsporon audouini*, *M. felineum*, and *Trichophyton gypseum* and scutula of *Achorion schoenleini* were placed, without any nutrient medium, in van Tieghem cells at various humidities controlled by osmotic solutions of known vapor pressure. These fungi, which while parasitizing the animal body produce hyphae and thallospores only, were then found to undergo a second period of growth on the detached infected host tissue in moist atmospheres. During this phase they produced all the highly differentiated spore forms previously known only on various media. The aerial hyphae, aleuriospores, fuseaux and spirals produced by such *in situ* cultures are described in some detail. It is suggested that in nature the saprophytic phase is initiated when the infected tissues fall from the animal body in a moist situation, and that this phase may have significance in the epidemiology of the disease.

Introduction

At least 180 species of dermatophytes have been mentioned in the literature as pathogenic to man. However, there is not a single species whose biology has been adequately investigated; in particular the significance of many of the structures found in the life cycle of these fungi is entirely unknown. The studies described in this paper were undertaken in an attempt to correlate structure with life cycle in the dermatophytes, and the paper forms an extended account of work which has already received preliminary notice in Nature (3).

The dermatophytes, in the narrow sense in which the word is used in the present paper, comprise those skin inhabiting fungi which cause favus and ringworm in its various manifestations. The dermatophytes form a physiological group of fungi capable of attacking such keratinized structures as the stratum corneum, hair, nails, horns, hooves and feathers. It is now generally believed on morphological grounds that, in addition to forming a physiological group, they are related taxonomically. The evidence presented in this paper tends to support this view.

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Contribution from the University of Manitoba Medical College, Winnipeg, Canada, with aid of a grant from the Banting Research Foundation, Toronto, Ontario.
[2] *Lecturer in Dermatology, University of Manitoba.*
[3] *Research worker on Dermatophytes, University of Manitoba.*

The ringworm fungus while infecting the hair consists of: (i) hyphae running longitudinally within the hair sheath or the shaft of the hair; and (ii) small round chlamydospores formed by the rounding-off and disarticulation of the hyphae, and therefore to be regarded as oidia or thallospores. The structure of the principal dermatophytes in their pathogenic phase has been dealt with amply by Sabouraud (8). It is a phase of limited structure, characterized by the production of immense numbers of thallospores, or in favus of compact masses of hyphae known as scutula.

On the other hand when growing on artificial media most dermatophytes produce conidia. Instead of the thallospores, differing little from one species to another, which characterize the dermatophytes in the hair, we find a variety of more highly differentiated structures which are often characteristic for different species. The morphology of the dermatophytes in culture has also been dealt with by Sabouraud (8) and various subsequent writers. Among the structures produced in culture may be mentioned: (i) the thick walled fuseaux or macroconidia of *Microsporon felineum*, which in shape somewhat resemble the conidia of a Helminthosporium; (ii) the aleuriospores or conidia produced on branched conidiophores by the majority of the dermatophytes; and (iii) the helically twisted hyphae or spirals found in cultures of members of the Trichophyton gypseum group. These conspicuous organs are well formed and often abundant in cultures, yet they are not observed while the fungus is living on the animal body. It is difficult to believe that such highly differentiated structures as those enumerated above are artifacts due to the unusual conditions of the culture tube, or that structures so regular in form and abundantly produced are merely atavisms. Most investigators have recognized that these organs point to the existence of a saprophytic phase in the life history of the dermatophytes yet undiscovered in nature.

Brocq-Rousseu, Urbain and Barotte (1) grew *Trichophyton gypseum* on the sterilized litter and fodder of stable animals, and reported that in the saprophytic condition the vitality and virulence of cultures on straw were retained for over two years. Langeron and Milochevitch (6) investigated the microscopic structure on such media and discovered that natural substances such as straw and grains of cereals are exceptionally favorable to the production of the more complex dermatophyte organs. They accordingly expressed the opinion that the life cycle of these fungi includes a phase on materials relatively poor in nutriment. The writers have adopted a somewhat different hypothesis, but one which, however, by no means excludes the view of the saprophytic phase expressed by Langeron and Milochevitch.

The evidence from cultures for the existence of spore forms and other structures in the dermatophytes which have never been found in nature formed the starting point of the enquiry.

The most obvious clinical feature of ringworm, both in man and animals, is the penetration of hair and epidermis by the fungus, followed by the falling of infected hairs and scales from the body. Ringworm is thus characterized by defluvium in hairy regions or desquamation of the glabrous skin. In

animals, less frequently in civilized man, this detached infected material must often fall into moist places such as among vegetation or humus. Arrived there it might either colonize the substratum upon which it finds itself, as assumed by the Parisian school, or on the other hand might continue growth under the new conditions by the absorption of water by the fungus present in the detached hair, scale or scutulum, without the necessity of colonizing new material.

As far back as 1902 Plaut (7) placed trichophyton-infected hairs in a moist environment and observed the development of mycelium and "ectospores". This important experiment appears to have been lost sight of by subsequent workers, and its significance in the life history of the ringworm organisms has never been appreciated. This work of Plaut was unfortunately not known to the writers when the preliminary communication was made of their investigation along the same lines, or reference would have been made to these interesting experiments which were carried out over 30 years ago.

Methods and Material

Experiments were designed to test the hypothesis that upon naturally infected hairs, scales or scutula detached from the lesions on the body the fungus could continue growth and produce spores. A few tests were sufficient to demonstrate the correctness of this hypothesis, and the following technique was developed during the course of several experiments. The object of the technique is to supply the fungus present in the infected material solely with water by exposing it to a moist atmosphere, and to withold all nutriment except that present in the infected tissue. Further, it was desired to expose the fungus to a range of humidities. This was achieved by placing the infected material in water vapor at various degrees of saturation.

The apparatus used was a slight modification of the van Tieghem hanging-drop cell. Each cell was set up in the following way. A clean flamed glass microscope slide 3 by 1 in. was laid flat on the table. The two cut ends of a sterilized glass ring, 18 mm. diameter by 10 mm. high, were dipped into melted white vaseline and quickly withdrawn, and the edges of the ring thus became coated with vaseline. The ring was then lowered on to the middle of the slide before the latter had completely cooled. The heat of the slide melted the vaseline in contact with it on the ring, and on further cooling a water tight union was formed between the slide and the ring. About 1 cc. of the solution determined upon to keep the atmosphere moist was then placed in the bottom of the cell by means of a pipette. Cover slips were carefully cleaned before use by boiling in alkali, rinsing in running tap water and wiping on a clean cloth. They were stored in alcohol until required. This was done to ensure that no soluble substances remained on the glass from which the fungus could derive nutriment or which might inhibit germination. Thus all growth was derived from food in the hair or scale. The cell was closed by lowering a clean, flamed cover slip on to the top of the ring and pressing it down into the vaseline deposited there.

The cell thus prepared was set aside for a few minutes to cool before being inoculated. As the cell cooled a film of water usually condensed like dew on the underside of the cover slip. This was used to attach the infected material to the cover slip. The cover slip was lifted off from the ring and the infected material quickly deposited in the water-film with sterile forceps or needle, and the cover slip replaced at once before the film had time to dry. Except in cells containing a saturated atmosphere the film usually evaporated again after a short time when equilibrium was reached in the cell, leaving the material reasonably securely attached to the cover of the cell. In the driest atmospheres employed in the experiments an adequate film sometimes failed to form. In these cells a small drop of sterile water was placed on the cover slip and the material placed in this before closing the cell. When the cell was closed the drop rapidly evaporated as before.

It was desired to place the hairs and other material under different humidities. The fact that the presence of a dissolved substance lowers the vapor tension of a liquid was utilized to supply various humidities to which the infected material was submitted. Sucrose was chosen as a solute with which to lower the vapor tension of water, as being a non-volatile substance easily weighed out, and highly soluble in water. Its non-volatility eliminated danger of sucrose molecules escaping from the liquid and serving as nutriment to the fungus in the infected tissue.

To prevent the growth of micro-organisms the humidifying solution also contained 1% of hydrated copper sulphate. This also served to remove the possibility that the fungus which grew out of the infected tissue might utilize the osmotic solution as a source of food by means of the long pendant aerial hyphae which are sometimes produced. Sucrose had the advantage over dextrose of not precipitating the copper sulphate added to the solution as a preservative.

The concentrations of sucrose which were principally employed to lower the vapor pressure over the solution were: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 molar. The approximate osmotic pressures and corresponding percentage humidities of the atmosphere in contact with solutions of various concentrations is shown in Table III. Throughout the paper the approximate humidities in the cells are expressed in percentage, and in each case the molarity of the sucrose solution used to produce the given humidity is shown in brackets.

The cells were left at the temperature of the laboratory, which remained within a few degrees of 23° C. during the period in which most of the experiments were conducted. Sudden falls in temperature of the room in which the cultures are kept are to be avoided as the atmosphere in the cells may become temporarily saturated before equilibrium is restored. Under these conditions water is deposited on the surface of the cultures, often destroying the natural arrangement of the organs.

For daily examination of the preparations a high power dry objective with a long working distance has been found useful, as deeper parts of the culture

can then be safely examined without danger of the objective touching the cover slip. To prevent heating of the cultures a heat absorbent was placed between the source of light and the condenser of the microscope. In the lower humidities, at least, the hyphae were in air, consequently it was impossible to make out details of the structure, such as septa, which were often invisible until the mycelium was mounted in some refractile medium.

The following technique has been found useful in rapidly making permanent preparations. The cover slip was removed from the cell, inverted, and a drop or two of glacial acetic acid run over the culture to fix it. While in the acetic acid most of the vaseline was wiped off the cover slip with a cloth. After five minutes the cover slip was placed with the culture downwards on the surface of a vessel containing distilled water. After several washings in distilled water over a period of three to four hours the culture while still wet was lowered on to a drop of melted glycerine jelly containing methylene blue on a slide.

In the preparation of the cells for the experiment sterile precautions were taken throughout. The hairs, scales and scutula, on the other hand, were placed in the cells just as they were taken from the body, without any attempt at sterilizing the outside of the material, although they were handled with sterile instruments in order to avoid contamination after being removed from the body. As would be expected under these conditions contamination of the cultures by saprophytic organisms was found occasionally. Bacteria appeared only when the atmosphere in the cell was moist enough to deposit water around the infected material, that is, in a saturated or nearly saturated atmosphere. Their occurrence is dealt with below. Saprophytic molds were found to develop at any of the humidities tested. Their occurrence was however exceptional, and the mold principally encountered was, as would be expected, *Penicillium*. Although contaminants occurred in a small percentage of cultures they were not frequent. Their occurrence however calls for a knowledge of the structures of the dermatophytes in pure culture before conclusions are drawn from this technique.

The technique is easy to carry out and is eminently suited for class demonstration. Students could make their own preparations and study the structure of dermatophytes direct from clinical material without the necessity of maintaining cultures on Sabouraud's medium. For class or routine laboratory purposes a molar sugar solution with 1% copper sulphate would probably be most generally useful.

The writers have borrowed Plaut's term "*in situ* cultures" for preparations of the dermatophytes obtained by this new technique. According to the nature of the inoculum *in situ* cultures comprise: infected-hair cultures, infected-scale cultures, and scutulum cultures.

Naturally infected tissue, consisting of hairs, scales or scutula, was taken from patients and stored in the laboratory between sterilized glass slides in small envelopes. The fungus present in each specimen was first determined by means of cultures on proof agar, prepared with the maltose *brute de Chanut*

and peptone *granulée de Chassaing* as specified by Sabouraud. Other portions of the material, which thus contained fungi already identified, were used to inoculate the moist cells.

A large number of *in situ* cultures were prepared with: (i) hairs from seven children with scalp ringworm due to *Microsporon audouini;* (ii) hairs from three children with scalp ringworm, and from two kittens with ringworm, due to *Microsporon felineum;* (iii) hairs from two children with kerion celsi due to *Trichophyton gypseum;* (iv) hairs and scutula from three children with favus capitis due to *Achorion schoenleini.*

The four common dermatophyte species studied have been described in the parasitic phase and in culture on various media by previous writers. The following descriptions of *in situ* cultures are intended to supplement the published descriptions of these fungi with an account of their morphology in the saprophytic phase on the host tissue. The descriptions are from notes and drawings made while the cultures were growing, and also from a study of preparations mounted in glycerine jelly. All the photographs are from living preparations in air.

Microsporon audouini

Microsporon audouini Gruby. *In situ* cultures: *aerial hyphae,* somewhat sinuous, diameter 1.5–3 μ; *aleuriophores* (conidiophores), long, erect, usually unbranched; *aleuriospores* (conidia), hyaline, unicellular, borne singly on lateral wall of hypha, sessile or on short necks, pyriform to oblong, proximal end faceted, distal end round, about 3–5 $\times$ 1.5–2 μ; *fuseaux* (macroconidia), sparse, hyaline, smooth, wall somewhat thickened, cylindrical to clavate, proximal end faceted, distal end round, 2- to 4-septate, 30–70 $\times$ 6–10 μ; *spirals* absent.

As shown in Table I the time required for the fungus in the infected hair to germinate was found to depend upon the humidity of the atmosphere in the moist cell. The germ tubes which emerged from the spore sheath around the hair were narrow sinuous hyphae when produced in a saturated atmosphere, but appeared straighter under drier conditions.

TABLE I

RELATION BETWEEN HUMIDITY AND GERMINATION

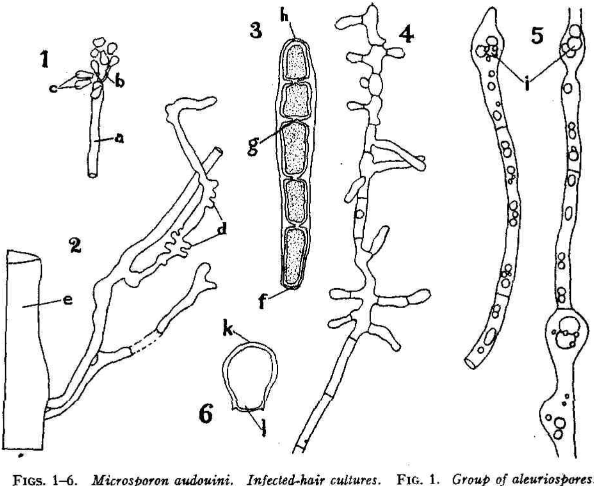
Conc. of humidifying soln., moles per litre	0.0	0.5	1.0	1.5	2.0
Germination time, days	1	2	5	10 Very poor	No germination

Aerial mycelium, which was well developed in cells of 98% humidity (1.0 *M*)*, consisted of straight or sometimes slightly sinuous hyphae of uniform diameter 1.5–3.0 μ wide, septate at intervals of 20 to 80 μ. The

**The figures in brackets show the molarity (M) of the sucrose solution used to produce the humidity indicated.*

aerial hyphae were sparingly branched, usually midway between two septa and at right angles to the parent hypha. The tips of the young aerial hyphae were often seen bearing conspicuous drops of liquid. Numerous hyphal fusions commonly occurred in the aerial mycelium after a few days.

In the saturated atmosphere over the 0.0 *M* humidifying solution, no conidia were observed and the mycelium, which remained in the water-film which accumulated around the hair, appeared abnormal. Branching was irregular and the hyphae were contorted and had irregular denticulate



FIGS. 1–6. *Microsporon audouini. Infected-hair cultures.* FIG. 1. *Group of aleuriospores:* a, *living portion of aleuriophore;* b, *part of aleuriophore whose collapse has massed together the aleuriospores* c. FIG. 2. *Pectinate hyphae,* d; *arising from portion of infected hair,* e. FIG. 3. *Fuseau in glycerine jelly, shown in optical section;* f, *faceted end;* g, *pore in septum;* h, *rounded apex.* FIG. 4. *Aleuriophore with short branches.* FIG. 5. *Senile hyphae containing oil drops, i.* FIG. 6. *Diagram of aleuriospore:* k, *rounded apex;* l, *faceted proximal end. Magnifications: No. 1,* × *485; Nos. 2–5,* × *1100. Humidities: Nos. 1 and 4, 99% (0.5 M); Nos. 2 and 5, 100% (0.0 M); No. 3, 98% (1.0 M).*

projections giving them the appearance, as shown in Fig. 2, of Sabouraud's pectinate organs. Portions of the hyphae behind the tips sometimes became evacuated. As this water-submerged mycelium aged, certain of the cells enlarged to form chlamydospores; usually however the wall remained unthickened, and finally after about eight weeks both the unaltered mycelial cells and the chlamydospores lost their granular protoplasmic appearance and came to contain merely a few spherical refractile bodies. Chlamydospores in this stage sometimes had a superficial resemblance to asci containing as-

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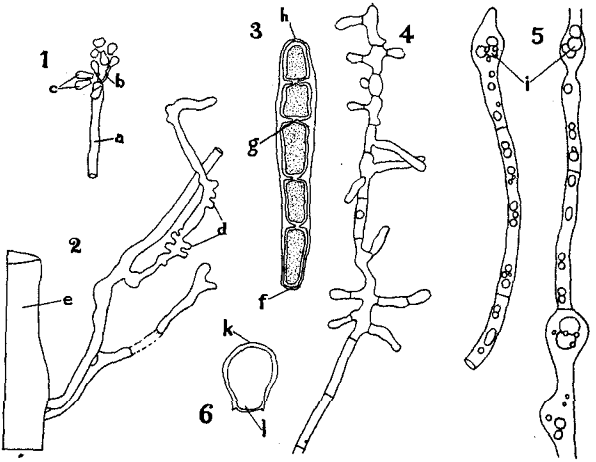

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cospores. Perhaps these structures are identical with the "askusähnlichen organen" described by Kambayashi (5) in aged liquid cultures of *Microsporon japonicum*. That the globular structures formed in the old infected-tissue cultures of *M. audouini* were really intracellular fat droplets and not ascospores is evidenced by the following properties: (i) the number of spherical bodies varied from one to many in a cell; (ii) their diameter varied from 0.6 μ to 4.3 μ; (iii) when examined with the oil immersion lens the bodies appeared homogeneous and showed no signs of division into cell wall and cytoplasm; (iv) the bodies did not stain with protoplasmic stains such as methylene blue but took up fat stains such as scarlet red avidly; and (v) so far from being confined to special enlarged organs they occurred in any cell of the aging mycelium (Fig. 5).

Aleuriospores (conidia), were borne on long, straight, aerial hyphae usually unbranched but sometimes showing one or two short branches at right angles as shown in Fig. 4. The aleuriophores were undifferentiated from the ordinary hyphae except that in the sporogenous portion of the hypha the septa were closer together. The hypha, emptied by the formation of the spores, tended to collapse thus bringing the spores together in an irregular mass as shown in Fig. 1.

The aleuriospores (conidia) were first noticed about seven days after germination of the fungus in the hair (Plate 1, Fig. 1). They differ from the spores produced in the hair while on the body in that they are borne singly on the lateral walls of the conidiophores and are sessile or on short necks at right angles to the aleuriophore. They are aleuriospores in the sense of Vuillemin, that is to say they are not constricted off from the parent hypha, but are cut off from the parent by a transverse septum at the base of the spore. They are liberated on mechanical contact or on the disintegration of the dead and emptied aleuriophore. They thus come to have a characteristic shape; the distal end is rounded as it is the apex of a hypha, but the proximal end is truncated or faceted, the facet representing the septum at the base of the spore (Fig. 6.). Aleuriospores (conidia) and fuseaux (macroconidia) when present, in all four species of dermatophyte studied were of this shape.

Fuseaux (macroconidia) were noted in a few cultures a day or two later than the aleuriospores but their occurrence in this species was infrequent. They were formed on aerial hyphae in the same position as the ordinary branches, that is, laterally near the middle of a vegetative cell which they left at right angles. The wall was thickened up to 1 μ, the thickening being greatest at the distal end of the fuseau (Fig. 3). The spore was divided by transverse septa into from three to five cells. The septa were perforated by a central pore as was seen by careful focusing on the septa when mounted in glycerine jelly. The mode of germination of the fuseaux was not observed. Both fuseaux and aleuriospores were produced at humidities slightly below saturation, namely, 99.5–100% (0.3–1.0 *M*). At 100% humidity (0.0 *M*) the mycelium appeared degenerate.

When infected-tissue cultures were transferred to proof medium the gross appearance of the resulting mycelia was typical of *Microsporon audouini*.

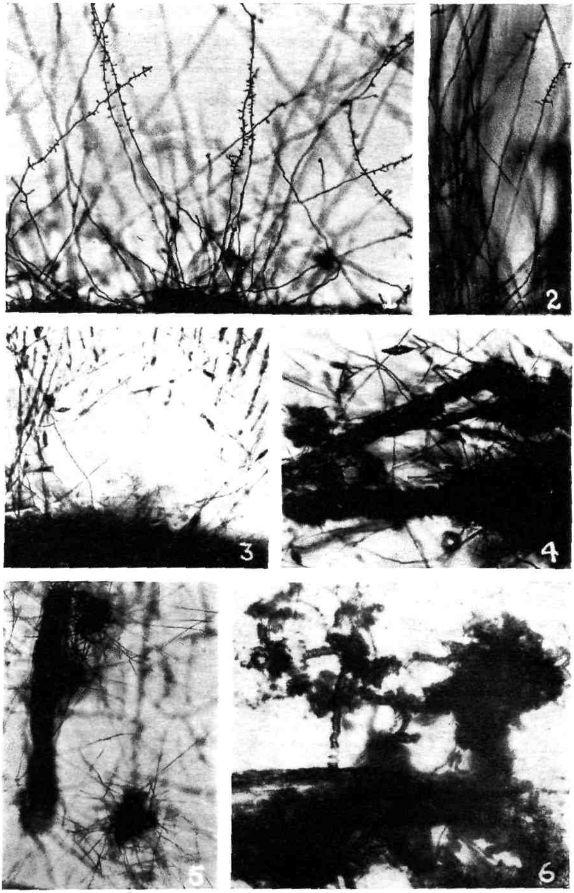
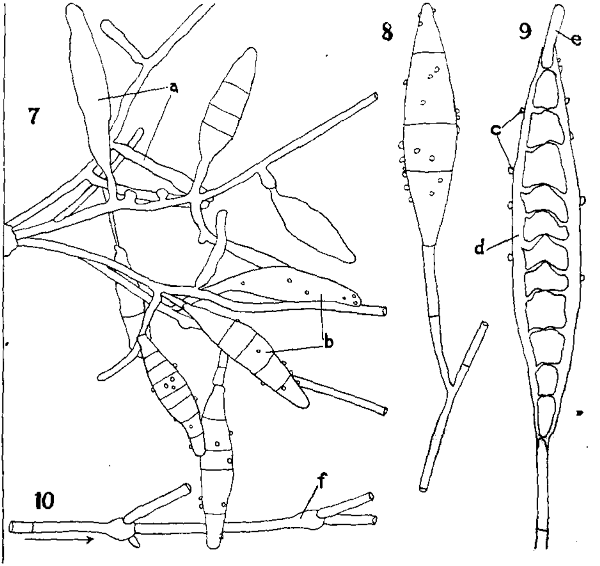


FIG. 1. *Microsporon audouini. Aleuriospores on aerial hyphae produced by infected human hair kept at 99% humidity (0.5 M), × 102.* FIG. 2. *Microsporon felineum. Aleuriospores on aerial hyphae from infected cat hairs kept at 99% humidity (0.5 M), × 102.* FIG. 3. *M. felineum. Fuseaux bearing apical drops of liquid, produced on mycelium from infected human hair kept in saturated atmosphere, × 47.* FIG. 4. *M. felineum. Fuseaux produced from infected cat hairs. The torulose surface is clearly visible, × 102.* FIG. 5. *Trichophyton gypseum. Developing spore bushes on mycelium produced from infected human hair kept in saturated atmosphere, × 61.* FIG. 6. *T. gypseum. Spirals produced from infected hair kept at 99% humidity (0.5 M), × 210.*

Microsporon felineum

Microsporon felineum Mewborn. *In situ* cultures: *aerial hyphae* straight, diameter 1–3.5 μ; *aleuriophores* (conidiophores), long, erect, usually unbranched; *aleuriospores* (conidia), sparse, hyaline, unicellular, borne singly and laterally, sessile or on short necks, pyriform to oblong, proximal end faceted, distal end round, 3–6 × 1.5–2 μ; *fuseaux* (macroconidia) abundant, terminal on short lateral branches of main hyphae, thick walled, hyaline or brownish, straight, fusiform, sparsely torulose, 3–11 septate, proximal end faceted, distal end round, 50–120 × 20–25 μ; *spirals* absent.

In a saturated atmosphere germination normally took place within 24 hr., but at lower humidities it was delayed (Table V). The germ tubes consisted of straight, even, relatively stout hyphae, which remained unbranched for some time. As in *M. audouini*, in saturated atmospheres, conspicuous drops



FIGS. 7–10. *Microsporon felineum. Infected-hair cultures.* FIG. 7. *Group of developing fuseaux as seen in air;* a, *young spores;* b, *older spores becoming torulose and septate.* FIG. 8. *Mature fuseau in air.* FIG. 9. *Mature fuseau in glycerine jelly:* c, *wart-like protuberances;* d, *region of greatest thickening of cell wall;* e, *germ tube emerging from apical cell.* FIG. 10. *Racket hyphae:* f, *swelling behind septum. Magnifications: Nos. 7 and 8,* × *485; Nos. 9 and 10,* × *1100. Humidities: No. 7, 98% (1.0 M); Nos. 8, 9 and 10, 100% (0.0 M).*

of liquid were excreted at the tips of the aerial hyphae. Sometimes the presence of these drops led to the production of loops in the mycelium, resembling those referred to by Sabouraud (8, p. 713) as pelotons. They appeared to be formed by the growing tip of the hypha being unable to escape, owing to surface tension, from the drop surrounding its tip and consequently becoming coiled during subsequent growth around the surface of the drop. These pseudo-spirals differ entirely from the true spiral structures which are met with in *Trichophyton gypseum* and are described below. Spirals, as observed by Langeron and Milochevitch (6) in cultures of *M. felineum* on oats, etc., have not been observed in infected-hair cultures.

The aerial hyphae were straight with uniform diameter which varied from 1 to 3.5 μ; the older hyphae were constricted slightly at the septa. The hyphae were septate at intervals of 20–60 μ. The main hyphae branched fairly freely. The majority of branches were formed just behind a septum and left the parent hypha at right angles but at once bent forward and came to lie at an acute angle with the parent hypha. Racket hyphae, in which branches arise from swellings behind the septa, characteristic of this species in culture were not found in the aerial mycelium, but were present on the glass cover of the cell in saturated atmospheres (Fig. 10). Abundant hyphal fusions were observed between aerial hyphae.

After some days aleuriospores were borne on long, straight, unbranched aerial hyphae (Plate 1, Fig. 2). In size and shape the aleuriospores resembled those of *M. audouini*. Their appearance in the infected-hair cultures was sporadic and much less frequent than fuseaux in this species.

Fuseaux were usually produced on the mycelium resulting from the infected-hair cultures at all humidities except those sufficiently dry to limit growth (Plate 1, Figs. 3 and 4). They were developed in from four to six days in a saturated atmosphere but were delayed considerably at the lower humidities. The occurrence of fuseaux was found to be somewhat sporadic and it was noticed that when the portion of hair placed in the cell was short no fuseaux were produced. Table II shows the relation observed in one experiment between the length of the infected portion of the hair and the occurrence of the fuseaux.

TABLE II

RELATION BETWEEN LENGTH OF INFECTED HAIR AND OCCURRENCE OF FUSEAUX

Length of hairs	Occurrence of fuseaux	
	Present	Absent
From 0.8 to 1.8	0 preparations	6 preparations
From 2.0 to 5.0	6 preparations	1 preparation

The fuseaux (Figs. 7-9) apparently represent modified lateral branches of the aerial hyphae. These modified branches consisted of one or two basal stalk cells which eventually collapsed, bearing a large, thick walled, spindle-shaped spore. Perforate septa divided the interior of the spore into from

about 4 to 12 cells. The length of the fuseaux varied from 50–120 μ, and the diameter from 20–25 μ. The size and degree of thickening of the wall was variable and appeared to depend upon the amount of infected material available to the developing mycelium. In some preparations the spores remained immature and thin walled and then somewhat resembled those of *Trichophyton gypseum*. The region of greatest thickening where the wall was sometimes 4 μ thick, coincided with the maximum girth of the spore. It was thinnest at the rounded apex, and at the cross wall of the hindmost cell where it had broken from the parent hypha. Germination usually took place through the thin wall of the apical cell and was sometimes observed while the fuseau was still attached to the parent hypha. The outside wall of the fuseaux was usually covered with minute colorless warty prominences which may be specially well developed on the apical cell. The mode of development of fuseaux and the origin of their septa as a ring-like ingrowth from the cell wall has been figured by Buller (2) from studies of infected-hair cultures made by one of the present writers.

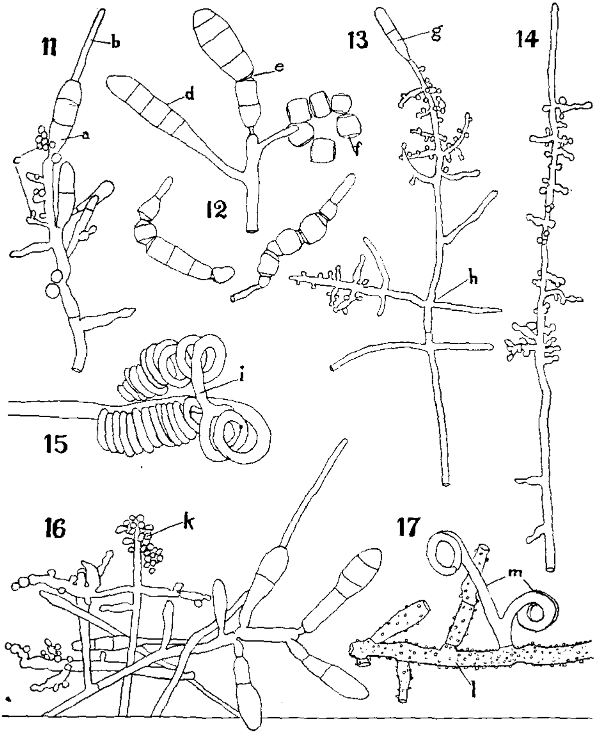
When the *in situ* cultures were transferred to Sabouraud's proof medium, typical mycelia of *M. felineum* were obtained.

Trichophyton gypseum

Trichophyton gypseum Bodin. *In situ* cultures: *aerial hyphae*, straight, diameter 1–3.5 μ, often cross branched; *aleuriophores* (conidiophores), long, erect, sparsely branched, or short much branched, and densely aggregated into white spore-bushes up to 1 mm. in diameter; *aleuriospores* (conidia) abundant, unicellular, hyaline, lateral sessile or terminal on lateral branches of hyphae, becoming densely aggregated by disintegration of parent hypha, proximal end faceted, distal end round, subspherical 2.8 × 2.5 μ, occasionally pyriform, 3–3.5 × 2.5 μ; *fuseaux* (macroconidia) often abundant, smooth, thin walled, hyaline, straight, clavate to cylindrical 4–10 septate, 40–75 × 7–10 μ, proximal end faceted, distal end round; *spirals* regular, counterclockwise, 1–30 turns, 8–12 μ in diameter, tightly or loosely coiled, sometimes branched, abundant in spore bushes; *torulose hyphae* present in spore bushes.

Germination of the fungus in hairs in a saturated atmosphere began within 24 hr. and was well advanced after 48 hr. For the first few days after germination the hyphae grew about 600 μ per day at a room temperature of 23° C. The mycelium consisted of unbranched straight hyphae varying from 1.0–3.5 μ in diameter, which did not show hyphal fusions until after the fourth day of growth when numerous fusions were observed in the aerial mycelium. Germination was slower at lower humidities and was not observed at humidities lower than 97% (1.5 *M*). The structures produced by the mycelium will be described at the humidity at which their development was most favorable.

The aerial hyphae branched at right angles to the parent hyphae, as did the species of Microsporon described above. A characteristic of *Trichophyton*



FIGS. 11–17. *Trichophyton gypseum. Infected-hair cultures.* FIG. 11. *Group of spores in air:* a, *fuseau and its germ tube* b; c, *aleuriospores.* FIG. 12. *Fuseaux six weeks old breaking down into chlamydospores;* d, *an intact spore;* e, *splitting of septum;* f, *fuseaux completely broken down.* FIG. 13. *Aleuriophore in air, with aleuriospores and small terminal fuseau; showing cross branching.* FIG. 14. *Aleuriophore in air.* FIG. 15. *Branched spiral from periphery of a spore bush, in glycerine jelly.* FIG. 16. *Sporogenous hyphae emerging from hair, showing mode of branching and position of fuseaux:* k, *group of aleuriospores collapsed on parent hypha.* FIG. 17. *Torulose hyphae bearing spirals,* m, *from interior of spore bush; mounted in glycerine jelly. Magnifications: Nos. 11, 12, 14 and 16, × 485; No. 13, × 315; Nos. 15 and 17, ×1100. Humidities: No. 11, 98% (1.0 M); Nos. 12 and 16, 99% (0.5 M); Nos. 13, 14, 15 and 17, 100% (0.0 M).*

gypseum, not observed in Microsporon, was the tendency for two rectangular branches to leave at opposite sides of the parent hypha. A characteristic rectangular appearance is then given to the mycelium (Figs. 13 and 16).

Unlike *Microsporon felineum*, in this species aleuriospores were produced regularly and in great numbers. But like the microsporons they were produced singly as round, unicellular, lateral bud-like outgrowths at right angles to the parent hyphae (Fig. 14), and were rounded at the distal end and faceted at the proximal end. They were smaller than the aleuriospores of the Microsporon species so far studied and more regularly nearly spherical. They varied from 2.8 to 3.5 μ in length by 2.5 μ in breadth and were almost sessile. The aleuriospores first appeared on the aerial mycelium about five days after germination had begun. In a saturated atmosphere they were at first borne at the ends of long delicate aleuriophores which differed from those of *M. audouini* and *M. felineum* in having a greater number of branches. Fig. 13 shows the tip of an aleuriophore bearing spores at a distance of one millimetre from the hair; the branches crossing the main hypha at right angles were characteristic of the mode of branching of the sporogenous hyphae of this species. The first formed aleuriospores were widely distributed in the aerial mycelium.

Later however the production of aleuriospores began to be concentrated into certain areas. At intervals in the mycelium, usually near the hair, dense bush-like masses of mycelium arose by the repeated rectangular branching of hyphae (Plate 1, Fig. 5). These no doubt corresponded to the so-called "sterile perithecia" of Langeron and Milochevitch (6). On the cover slips of the preparations they were visible to the naked eye as white points in the aerial mycelium. The hyphae composing these masses, which are here termed *spore bushes*, were stouter than the ordinary aerial mycelium, septate at shorter intervals, and branched in the usual rectangular manner of this species. In the interior of the spore bushes were produced great numbers of aleuriospores which, by the disintegration of some of the sporogenous hyphae, came to fill the space between the main hyphae of the structure without apparent order. No asci or ascospores have been observed at any stage of the spore bushes even after four months of growth in the cultures. Aleuriospores were most abundant at the higher humidities employed; under drier conditions fuseaux predominated. In the spore bush a rectangularly branched network of coarse torulose hyphae was often present (Fig. 17). Similar torulose hyphae were observed in this species in cultures on natural media by Langeron and Milochevitch. These hyphae did not bear spores but branched freely and frequently terminated in spirals. The warts appear to be of the same nature as the cell wall of the hypha, and do not resemble the collar-like projections left on a hypha after an aleuriospore has broken off.

Spirals usually appeared at about the same time as the spore bushes. At first they were scattered throughout the aerial mycelium, but later became more and more abundant and well developed around the spore bushes (Plate 1, Fig. 6). The spirals were in the form of three-dimensional, cylindrical

helices of from 1 to 30 turns, and consisted of very even, septate hyphae, about 1.5–2 μ in diameter, coiled into a spiral about 8–10 μ wide. The length of the spiral naturally depended on the number of turns and the distance between each successive turn of the coil. The spirals were very regular in form. They were counterclockwise structures, sometimes branched as illustrated in Fig. 15. As the structure and significance of these spirals will be considered in another communication, it is here sufficient to record that in the infected-tissue cultures they were abundant, exceptionally well developed, and exactly resembled those formed in culture on various media.

Fuseaux, unlike aleuriospores in this species, were more abundant when the humidity was low. Plate 2, Fig. 1, shows a naturally infected hair surrounded by a halo of fuseaux after six weeks at 98% humidity (1.0 *M*). The best development of fuseaux was observed at 98% humidity (1.0 *M*). The fuseaux of *T. gypseum* differed from those of *M. felineum* in shape and also in that the wall was relatively thin. They were club shaped bodies up to 70 μ long and 10 μ in diameter with the proximal end faceted. They were divided by perforate septa into from 4 to 10 cells and germination often took place through the apical cell while the spores were still attached to the parent hypha. The arrangement and relative sizes of the aleuriospores and small fuseaux is illustrated in Figs. 11 and 16. In cultures two months old fuseaux situated on the cover slip of the preparation were found to have undergone a secondary change. Each cell of the spore became enlarged, the septum between adjacent cells split and the cells separated as if by mutual pressure as shown in the drawing reproduced as Fig. 12. Thus each fuseau came to be divided up into a number of chlamydospores.

When infected hair cultures were transferred to Sabouraud's proof medium, characteristic powdery colonies of *T. gypseum* were obtained.

Achorion schoenleini

Achorion schoenleini (Lebert) Remak. *In situ* cultures: *aerial hyphae* sparse, erect, straight or sinuous, branching at right angles, or apparently dichotomous; *aleuriospores* (conidia) borne laterally on erect hyphae, hyaline, unicellular, sessile, pyriform, proximal end faceted, distal end round, 3–5 × 1.5–3 μ; *fuseaux* (macroconidia) clavate, poorly differentiated from mycelium; *spirals* absent.

When infected hairs from patients with favus were put in a saturated atmosphere germination occurred at only one or two points along the hair. Mycelial development was very poor, the farthest branches reached only about 100 μ from the hair. All the hyphae were confined to the film of water which at this humidity accumulated around the hair. The mycelium consisted of somewhat irregular, coarse, branched hyphae which soon formed cyst-like lateral and intercalary chlamydospores, (Fig. 18) and no further development was noted on favus hairs.

When, instead of hairs, scutula were placed in the moist chambers further development took place, perhaps in consequence of the greater supply of

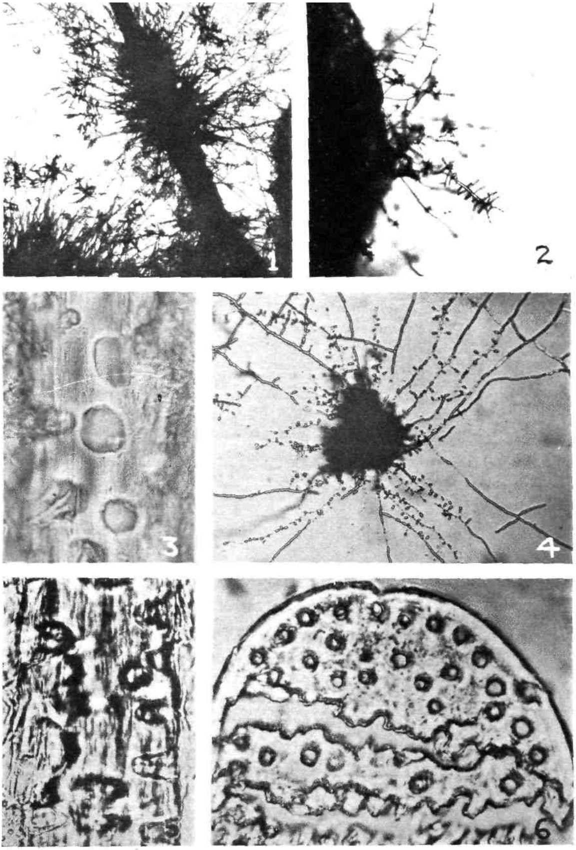


Fig. 1. *T. gypseum. Halo of fuseaux on mycelium growing from infected hair kept in saturated atmosphere,* × *45.* Fig. 2. *Achorion schoenleini. Aerial sporogenous hyphae developing from scutulum kept at 98% humidity (1.0 M),* × *102.* Fig. 3. *Saprophytic invasion of healthy human hair when placed for five days on surface of culture of Microsporon felineum on Sabouraud's agar. Surface of hair in focus, showing openings of wide rounded pits,* × *400.* Fig. 4. *A. schoenleini. Aleuriospores on hyphae adhering to glass cover of cell, developing from fragment of scutulum kept at 98% humidity,* × *150* Fig. 5. *The same as Fig. 3, but with axis of hair in focus, showing rounded bottoms of pits suggestive of enzymatic erosion,* × *400.* Fig. 6. *Transverse section human hair naturally infected with Achorion schoenleini, showing longitudinal canals in section,* ×*1050.*

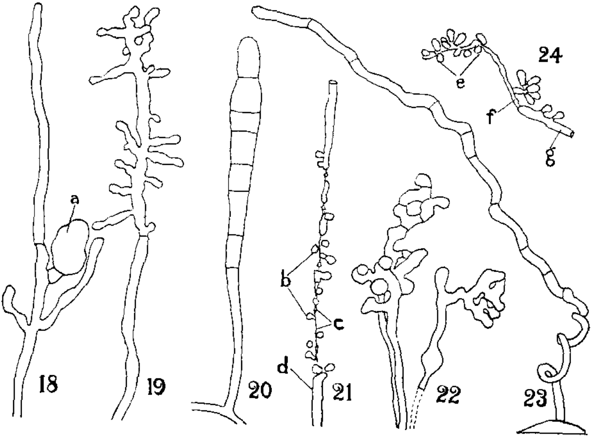
food present in a scutulum. In a saturated atmosphere (100% humidity (0.0 *M*)) some degree of bacterial contamination was always present, but it was never found to check completely the growth of the fungus from the scutulum. At this humidity the scutulum was always observed to accumulate around itself a considerable film of water, and all hyphae remained in the film. Germination began in about 24 hr. and after a few days the scutulum was surrounded by a fringe about 0.1 mm. broad of fairly straight, sparsely branched hyphae filled with granular protoplasm. The ends of the hyphae then underwent exceedingly irregular growth as illustrated in Fig. 22. The older parts of some of the hyphae then became emptied and terminal and intercalary chlamydospores were formed but no further development took place.

To obtain characteristic dermatophyte structures in *Achorion schoenleini* it was necessary to submit the scutula to considerably drier atmospheres. Germination was obtained in humidities as low as 93% (3.0 *M*) but the best growth was obtained at about 98% (1.0 *M*) at which humidity no bacteria developed and no free water was visible around the scutulum. Germination at 98% humidity (1.0 *M*) began in little more than a day and proceeded, as at 100% (0.0 *M*), until the scutulum was fringed with aerial hyphae. Extensive mycelium never developed in this species as it did in the dermatophytes referred to above, and to the naked eye the only visible change was a velvety appearance acquired by the scutulum. Some of the hyphae tended to spread over the cover slip in complex arborescent branching systems, which rendered observations on deeper lying structures difficult. It is remarkable that no hyphal fusions were observed in the mycelium of this species. In this respect *A. schoenleini* resembles *Trichophyton album* in cultures of which the writers have been unable to find hyphal fusions. On the other hand *A. schoenleini* differs from the other three species described in this paper which form abundant hyphal fusions in the aerial and submerged mycelium. As early as the fifth day after germination some of the aerial hyphae acquired rectangular cross-branches resembling those of the sporogenous hyphae in *T. gypseum* (Plate 2, Fig. 2; and Fig. 19). Aleuriospores were formed on these hyphae, and also on unbranched hyphae represented in Fig. 21. As in the three species described above the aleuriophore becomes emptied of protoplasm during the formation of spores as shown in Plate 2, Fig. 4; and Fig. 24. The aleuriospores were mostly pyriform rather than spherical, in this respect differing from those of *T. gypseum*.

Long club-shaped hyphae were common in the aerial mycelium, and these are regarded as fuseaux (Fig. 20). They were, however, rudimentary and not well differentiated from the ordinary mycelium. Thus scutulum cultures of *Achorion schoenleini* developed the characteristic dermatophyte fuseaux which were unknown until 1929 when Sabouraud and Negroni (9) observed them in cultures on a special medium containing honey. Sabouraud's belief that *Achorion schoenleini* is related to the more typical dermatophytes is thus supported by the occurrence in all four species of aleuriospores and fuseaux on the host tissue.

True spirals resembling those of *T. gypseum* were lacking, but a tendency to spiral twisting of the hyphae was of frequent occurrence in the aerial mycelium. One of these hyphae is illustrated in Fig. 23. Whether they have any relation to true spirals is doubtful.

When *in situ* cultures were transferred to Sabouraud's proof medium, typical mycelia of *A. schoenleini* were obtained.



FIGS. 18–24. *Achorion schoenleini. Scutulum cultures.* FIG. 18. *Vegetative hyphae produced in saturated atmosphere, with chlamydospore,* a, *on short lateral branch.* FIG. 19. *Aleuriophore drawn in air.* FIG. 20. *Rudimentary fuseau.* FIG. 21. *Part of sporogenous hypha;* b, *aleuriospores;* c, *remains of emptied hypha;* d, *living portion of hypha.* FIG. 22. *Portions of arborescent hyphae produced in saturated atmosphere.* FIG. 23. *Twisted hyphae.* FIG. 24. *Tip of aleuriophore:* e, *aleuriospores;* f, *collapsed portion of hypha;* g, *living hypha. Magnifications: Nos. 18, 20 and 22,* × *485; Nos. 19, 21, 23 and 24,* × *1100. Humidities: Nos. 18 and 22, 100% (0.0 M); Nos. 19, 21, 23, 24, 98% (1.0 M); No. 20, 96% (2.0 M).*

Viability of the Organisms

Moist cells were set up with hairs epilated recently and also with hairs stored for up to 28 months after removal from the patient. The parasite was found to remain dormant but viable for many months after detachment from the body. However, the specimens were stored under dry conditions in a laboratory where the relative humidity may fall as low as 10% during the winter, and material exposed to other atmospheric conditions in nature would not necessarily give the same results.

Microsporon audouini. Germination was obtained regularly from hairs stored for 12 and 15 months, but specimens tested after 27 months' storage failed to grow.

Microsporon felineum. Infected hairs removed from human patients and tested after 28 months did not germinate, but growth was observed from hairs removed from another patient 27 months previously.

Trichophyton gypseum. This organism was found to retain its viability in hairs for at least eight months after epilation.

Achorion schoenleini. Scutula from cases of favus capitis were viable for at least a year.

Incidentally it was noted that hairs infected by *Microsporon* which failed to germinate and in which the fungus was presumably dead, fluoresced in ultra-violet light as strongly as hairs recently epilated. It therefore appears that the life of the fungus is not a necessary factor in the phenomenon of fluorescence.

Effect of Various Humidities

Table III shows the relation between the molar concentration, osmotic pressure, and percentage saturation of the atmosphere in contact with the humidifying solutions, as calculated from data given in International Critical Tables (4) for temperatures from 20°–25° C. The 1% of copper sulphate contained in the solution is disregarded in these calculations which must be regarded as approximate only.

TABLE III

RELATION BETWEEN CONCENTRATION, HUMIDITY AND OSMOTIC PRESSURE

Conc. of humidifying soln., *moles per litre*	0.0	1.0	2.0	3.0
Humidity of atmosphere, % saturation	100.0	98.1	95.8	93.3
Osmotic pressure, atm.	0	26	51	77

The effects upon the development of the organisms of the various humidities to which the infected hairs and scales were exposed can be dealt with under the following headings: (i) effect on bacterial contamination; (ii) effect in limiting germination; (iii) effect upon the growth rate of the mycelium.

(i) *Bacterial contamination.* Contamination of the infected-tissue cultures by saprophytic molds occurred occasionally in cells at any of the humidities employed. Bacteria were seldom found in tissues infected by *Microsporon audouini* or *M. felineum.* Hairs infected by *Trichophyton gypseum* or scutula of *Achorion schoenleini* usually became contaminated by staphylococci during the first day or two if the atmosphere in the cell was nearly saturated with water vapor (100 to 99.5% humidity (0.0 to 0.3 *M*)). At humidities lower than these bacteria did not grow. It appears that the fungi are able to grow under much drier conditions than these bacteria, a fact which may be useful in isolating fungi from material which is bacterially contaminated. Of the four dermatophytes studied, that with the narrowest range of humidity tolerance grew over solutions of from 0.0 to 1.5 *M*, while the bacteria present were checked in the absence of free water. Often the cocci developing around the diseased material of *T. gypseum* were rapidly checked by the growth of the fungal hyphae, while the hyphae did not appear to be permanently affected by the contamination.

(ii) *Limiting germination.* Although the dermatophytes were capable of growing under considerably drier conditions than the bacteria encountered, a comparison of Tables III and IV will show that even for them a very high percentage of atmospheric moisture is necessary if they are able to imbibe enough water from the air to make germination and growth possible.

TABLE IV

HIGHEST CONCENTRATION OF HUMIDIFYING SOLUTION OVER WHICH GROWTH WAS OBSERVED

Species	Limiting concentration, molarity
Microsporon felineum	2.0
Microsporon audouini	1.5
Trichophyton gypseum	1.5
Achorion schoenleini	3.0

From a comparison of Tables III and IV it will be seen that the fungi present in infected tissue are able to imbibe water from the atmosphere over a humidity range of only about 5% below saturation. So long as the fungus is growing the fungus cell must have imbibition forces sufficiently great to remove water vapor from the atmosphere. At the lowest concentration of the humidifying solution over which the fungus is unable to imbibe water both fungus and solution must be in equilibrium with the water vapor in the atmosphere of the chamber. This is taken to indicate that the suction force of the fungus cells is greater than the osmotic pressure of the solution of the highest concentration over which growth is possible. This is a modification of the principle involved in Barger's well known method of comparing the osmotic pressures of two solutions. Such are the forces available to the fungus for drawing water out of the air below the saturation point or from the soil with which it is in contact.

(iii) *Rate of growth of the mycelium.* Radial growth of the mycelium was most rapid in cells containing atmospheres of the higher humidities. In cells with a saturated atmosphere, the hyphae became immersed in the drop of liquid which accumulated around the tissues, or else became irregularly branched owing to the surface tension of the liquid. Again at the higher humidities it was sometimes retarded by bacterial contaminants. Better growth was therefore often obtained at humidities slightly below saturation. In one experiment (Table V) careful measurements were made with the micrometer eyepiece of the daily growth at various humidities from hairs infected by *M. felineum*. Each humidity was represented by two preparations, and measurements were taken in two positions on each mycelium.

TABLE V

RADIAL GROWTH OF *Microsporon felineum* AT VARIOUS HUMIDITIES

Conc. of humidifying soln., *M*	0.0	0.5	1.0	1.5	2.0
Time of germination, days	1	1	1	2	4
Mean daily growth on 2nd and 3rd days, μ	1000	870	500	350	50*

*Only a few spores had germinated.

Discussion

The life history of the four common dermatophytes described above appears to be divided into two phases which are termed for convenience ***parasitic*** and *saprophytic*, although even in the lesion on the body the milder ringworm pathogens, such as *Microsporon audouini*, do little more than attack tissue already dead and keratinized.

The parasitic phase is passed on the animal body, where the keratin of the epidermis, and possibly the lymph, provides the organism with nutriment. The phase on the animal body is characterized: (i) by the formation of immense numbers of thallospores, or in *Achorion schoenleini*, of compact masses of hyphae known as scutula; (ii) by the longitudinal direction of the hyphae within the hair; and (iii) by the fact that each hypha within the hair lies in a narrow longitudinal canal which it completely fills. A cross section of a hair infected by *Achorion schoenleini* (Plate 2, Fig. 6) shows the longitudinal canals cut through transversely.

The saprophytic phase has long been known in culture. The four species tested by the *in situ* culture method were found to produce all the spore forms and most of the mycelial forms hitherto known only in culture on various media. They are therefore able to pass both the parasitic and the saprophytic phases on the host tissue alone, without the necessity of supplementary nutriment. From these experiments the saprophytic phase is believed to be initiated in nature under suitable conditions of humidity when the infected tissues become detached from the animal lesion, or possibly, if the infected animal dies, upon the dead body.

The saprophytic mycelium produces typical aleuriospores and fuseaux. Growth in the saprophytic phase appears to take place principally by the imbibition of water by fungal protoplasm which was formed during the parasitic attack of the tissue, and little by colonization of those parts of the hair or scale which remained unutilized. Portions of the tissue which were unattacked when the preparations were set up frequently remained unattacked for several weeks after the production of aleuriospores. Eventually, however, some colonization of the unattacked keratin took place, especially when the moist cells contained a saturated atmosphere. This saprophytic colonization of the hair, as described below, results in a type of attack differing entirely from that which characterizes attack during the parasitic phase. The saprophytic phase of the dermatophytes studied thus resolves itself into two stages: local growth resulting from imbibition of water by fungal protoplasm formed during the parasitic phase; and growth by colonization of fresh tissue. The characters of the two stages will be considered separately.

The stage of saprophytic growth by imbibition is characterized in *Microsporon audouini*, *M. felineum* and *Trichophyton gypseum*, by the germination of spores in the spore sheath and the establishment of hyphal fusions between the resulting hyphae, producing a radiating, branched, sporulating mycelium which may have a diameter of 1 cm. or more. During this stage the tissue previously infected is more thoroughly penetrated by hyphae. Growth of

the scutulum of *Achorion schoenleini* may be likened to the germination of a sclerotium, which becomes surrounded with a fringe of short sporogenous hyphae.

The stage of saprophytic colonization is characterized by: (i) the erosion of the hair in a radial direction by hyphae which lie in wide pits which they do not fill; (ii) by the rounded ends of these pits which strongly suggest that the keratin around the hyphae has been dissolved by enzymatic action; and (iii) the subsequent development of aleuriospores on the newly colonized portion of the hair. Exactly similar rounded pits were produced when uninfected hairs were laid upon the surface of living cultures of *Microsporon felineum* as shown in Plate 2, Figs. 3 and 5. When uninfected hairs in moist test tubes were inoculated with *Trichophyton gypseum* the same type of wide rounded pits was produced leading eventually to the more or less entire decomposition of the hair on which in turn were developed aleuriospores, fuseaux and spirals. The factors which determine the differences in the mode of attack upon the hair during the parasitic and saprophytic phases are at present unknown, but it is suggested that they are to be looked for in the highly specialized environment provided by the animal body, rather than in changes in the nuclear phase of the parasite. Asci have not been observed in infected-tissue cultures, even when preserved for six or eight months.

In nature the saprophytic phase is believed to be passed on detached host tissue. One fact which supports this hypothesis is that very few organisms are able to attack keratin. Considerations which support the hypothesis were discussed in the writers' preliminary note (3). "Although ringworm fungi will grow upon various sterilised natural media, such as horse-dung, straw, and grains of cereals, it has not yet been shown that they can flourish on these substrata under natural conditions which involve competition with other organisms. In the fallen infected hairs, on the other hand, a dermatophyte is in an advantageous position relatively to other organisms. By the time the infected hairs and scales fall from the body, the parasite has thoroughly penetrated their substance and converted much of it into fungal spores. A considerable amount of fungal protoplasm is therefore lying dormant within the hair ready to begin growth at once when brought into a suitable environment. Further, a fungus growing on such keratinised tissues as hair and scales has the advantage of freedom from competition, since it is growing on a substance which few other organisms are able to attack. It is possible that saprophytic stages of ringworm fungi may one day be found in Nature on various substrata other than fallen hairs. However, it may well be that cast-off hairs and epidermal scales are the most important natural substrata on which ringworm fungi pass their saprophytic existence and produce spores capable of infecting new human and animal hosts." Such a saprophytic phase in the life history of the dermatophytes must be of significance in the epidemiology of the diseases which they cause, especially in view of their long viability in infected tissue and ability to grow under drier conditions than bacteria.

The importance of the study of the microscopic structure of the dermatophytes must be emphasized. In the past, species have been based upon the gross appearance of the cultures on Sabouraud's media, especially upon texture, color and contour on these media. The microscopic organs have been believed to be variable and practically no attempt has been made to base species upon intimate studies of their morphology.

Naturally infected host tissue provides a relatively standard culture medium, upon which, under suitable conditions, mycelium aleuriospores, fuseaux and spirals develop. All the dermatophytes described above produce characteristic microscopic structures on host tissue by which the species can be distinguished. It is believed that when greater attention is paid to these structures the gross appearance of the colony will take its place as one of the several groups of characters by which these organisms may be classified.

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NOTES ON CERCARIAL DERMATITIS WITH DESCRIPTIONS OF THE CAUSATIVE ORGANISMS, *CERCARIA WARDLEI* N.SP., *CERCARIA BAJKOVI* N.SP., AND THE PARTHENOGENIC STAGE OF *CERCARIA ELVAE* MILLER[1]

By J. A. McLeod[2]

Abstract

This paper records an investigation of cercarial dermatitis, commonly known as *swimmer's itch* or *slough itch*, observed on bathers in Clear Lake, Manitoba. The infestation is manifest by the presence of erythematous papules and causes severe itching.

As a result of a survey for animal parasites in the lake, and experimental infestations with all forms it has been found that the disease may be caused by *Cercaria elvae* Miller, a brevifurcate distome cercaria, or by either of two pharyngeal longifurcate distome cercariae, *Cercaria wardlei* n. sp. and *Cercaria bajkovi* n. sp. Clinical and pathological notes on the disease are given.

Introduction

During the past 20 years numerous medical practitioners in Manitoba have been called upon to diagnose and prescribe treatment for a severe and unusual type of dermatitis which appears in the form of large erythematous papules which are intensely itchy. The fact that nothing of the nature of a bacterium could be cultured from extracts of these papules, and the observations that it is non-contagious and disappears spontaneously after a course of a few weeks, left considerable doubt as to the origin of the disease. In each case enquiry revealed that the affliction first made its appearance shortly after the patient had bathed in a body of water known as Clear Lake.

Numerous theories have been advanced regarding the origin of the disease. Some investigators have attributed it to the penetration of the pores of the skin by fine particles of sand. Others believed that it resulted from contact with poisonous plants growing on the shore, and some ascribed it to extreme individual susceptibility to toxic plant juices in solution in the water. Because of the failure of the disease to respond readily to treatment, such supposedly susceptible people were advised to refrain from further bathing in these waters.

Cases were not sufficiently frequent to warrant an extensive investigation until 1930 when the area surrounding the lake was converted into a national park. The increased number of campers and tourists visiting this resort resulted in an alarming number of cases of dermatitis. During the summer of 1933 over 55,000 people visited the lake and 50% or more of the bathers contracted the disease.

Past efforts on the part of two men, over a period of three years, to identify the causative agent of the disease have revealed nothing save that a fork-

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tailed organism was found in the water. Treatment of the water with large quantities of copper sulphate did not decrease to any extent the number of infestations.

The purpose of the investigation reported here therefore was to identify the causative organism and to determine the pathological condition produced and the duration of infestation. Knowledge of the life cycle with a view to bringing about efficient eradication of the parasite was also desirable.

An examination of the literature reveals the report of a similar disease by Cort (1). In this case *C. elvae* Miller was the causative organism and accidental infestation was produced by dipping the forearm into a pail of water containing parasitized molluscs. Further notes by the same author (2) state that a similar disease had been encountered in various parts of the United States, Haiti and France. In 1930 Matheson (4) reported cases from Cardiff in which *C. elvae* Miller was suspected. Similarly Taylor and Baylis (8) published the results of experimental work in Great Britain on a Cercaria which causes dermatitis. Again in 1930 Vogel (9) gave an account of cercarial dermatitis in Germany.

Procedure

Clear Lake, Riding Mountain National Park, is a body of exceedingly clear water about eight miles in length with a maximum width of three miles. The greatest known depth is 180 ft. and the bottom is of pure sand and coarse stones. Temperatures in June and July vary from a minimum of 0.9° C. in the deepest part to 29.5° C. in shallow water. Macroscopic aquatic vegetation is sparse and confined to localized patches near the shore.

A careful survey of the water in the vicinity of the bathing beach was made by means of fine plankton nets towed at various depths. Lower layers failed to yield any suspicious organism but the surface layer of about one foot in depth, on microscopic examination, produced the three species of furcocercous Cercariae listed below. Only living specimens were of any value for purposes of identification as contraction during fixation obliterated internal detail. Living specimens were lightly stained with dilute neutral red which penetrated the digestive tract and penetration glands. One minim of saturated chloretone in 30% alcohol added to 10 cc. of water containing the organisms was sufficient to anaesthetize active specimens without producing disintegration. Permanent preparations were made by fixing in hot 70% alcohol and mounting in glycerol jelly.

Eight species of gastropod molluscs were taken from these waters in an effort to determine the secondary host of the *Trematoda*. They may be briefly listed as follows:—*Limnaea stagnalis oppressa* Say.; *Limnaea obrussa* Say.; *Planorbis campanulatus* Say.; *Physa gyrina* Say.; *Volvata tricarinata* Say.; *Amnicola* sp., *Planorbis* sp.; *Planorbis trivolvis* Say.

Of these, only *Limnaea obrussa* and *Physa gyrina* were found to be infested. However both species are very plentiful over the entire lake from a foot above the water's edge to a depth of 4–5 ft. where they are found clinging

to the stones and vegetation. Large numbers of gastropods were gathered at different times for the purpose of determining the percentage of infestation. This was done by simply isolating each snail overnight in a test tube of water, which was then examined for cercariae, or by dissection of the snails. The results showed that 87% of all snails were parasitized.

The actual escape of cercariae from the mollusc host has not been observed closely but the rate is somewhat greater in darkness than in the light. Eight snails placed in 500 cc. of pure water for 14 hr. in darkness liberated 15,875 cercariae. These organisms are both phototactic and thermotactic and tend to congregate near the surface of the water or, if in a beaker, they gather at the side toward the light. If a finger be placed in a beaker containing cercariae, under a microscope the Trematoda will be seen to travel immediately towards the finger and repeatedly dart toward its surface in an effort to find an opening or grasp a hair.

Multiple infestations of molluscs were commonly observed during the survey but usually only a single species was obtained from the snails of one area. These areas varied in width from a few yards to several hundred, with an uninfested area between in some cases and overlapping in others.

All attempts to determine the primary hosts or obtain the adult stage of the parasites gave negative results. Five young and two adult white mice were placed in water containing all three species of cercariae for periods varying from 30 to 60 min. Post-mortem examinations of these one month after exposure failed to reveal any adult trematodes, although other investigators (6, 7) have been successful in producing infestations with other species.

Clear Lake is visited by a great many species of water birds but it was impossible to obtain any of them for examination. Post-mortem examination of four specimens of *Citellus tridecemlineatus* and one short-tailed shrew, *Balarina brevicauda talpoides*, showed them to be free from trematodes. Examination of the feces of specimens of *Lepus americanus* and *Fiber zibithicus* also from the shores of the lake gave negative results.

Taxonomy

The lack of any knowledge of the adult stage of the two previously unrecorded species necessitates the use of the larval name "*Cercaria*."

Classification of the species, based on the plan of Miller (5), is as follows:—

Pharyngeal brevifurcate distome cercaria, Group D:—*Cercaria elvae* Miller.

Pharyngeal longifurcate distome cercaria:—*Cercaria wardlei* n. sp.* and *Cercaria bajkovi* n. sp.**

Cercaria wardlei n. sp.

Type host. Limnaea obrussa.

Type locality. Clear Lake, Manitoba.

Specific diagnosis. A fairly large longifurcate pharyngeal distome cercaria about 770μ in length with a stout rectangular body. Furcae are slightly

**Named in honor of Professor R. A. Wardle, University of Manitoba.*
***Named in honor of Dr. A. D. Bajkov.*

longer than the tail stem and diverged while in the resting position. Anterior one-third of the tail stem and body is sharply turned to the left at an angle of 45° when the organism is suspended in the water. Three pairs of penetration glands are present posterior to the ventral sucker, which is one-third of the body length from the posterior end. The esophagus branches into two blind caeca which lie in the posterior body region. The anterior organ and ventral sucker bear numerous spines but an oral tuft of bristles is absent. Six pairs of flame cells are present in the body and one pair in the tail stem. Branches of the excretory tube open anteriorly in the mid-furcal region.

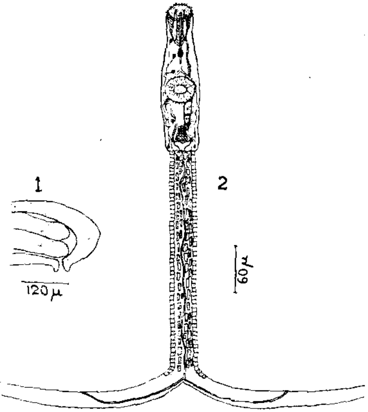

FIG. 1. *Cercaria wardlei, sporocyst showing birth pore.*
FIG. 2. *Cercaria wardlei showing internal structure.*

The average dimensions of moderately extended living specimens under a cover glass are:—body, 180 by 41μ; tail stem, 284 by 36μ; furcae, 306μ. Those of well extended fixed specimens in glycerol jelly are:—body, 144 by 27μ; tail stem, 274 by 27μ; furcae, 262μ. The anterior organ is one-fourth as long as the body, pyriform with the small end directed inward; two apparent head glands are present. The ventral sucker is a relatively large circular structure one-third of the body length from the posterior end and is slightly protrusible. The anterior organ and ventral sucker bear numerous retrorse spines and the anterior portion of the body is lightly spined. The tail stem is attached slightly ventrad to the posterior body termination; its surface is finely annulated and definite caudal glands are absent but it contains numerous small caudal bodies. From the periphery to a central fibre run numerous heavy bands of striated muscle in an oblique direction.

The mouth opening is subterminal; a pharynx is present around the narrow esophagus which bifurcates in the mid-body region into two large club-shaped caeca. These pass to within a short distance of the posterior end of the body. Three pairs of coarsely granular penetration glands are located posteriorly in the body between the germ cell mass and the ventral sucker. Their ducts are quite conspicuous and open dorsally and laterally to the mouth opening. Six pairs of flame cells are present in the body, disposed in each lateral half into an anterior and a posterior group of three, and

one pair in the tail stem. Branches of the excretory tube open anteriorly in the midfurcal region.

The parthenitae are long unbranched slightly motile sporocysts with almost uniform diameters of 170μ. Each contains numerous cercariae in various stages of development. The terminations are bluntly rounded, the birth pore being subterminal with conspicuous lips.

This larva may be readily distinguished from the other two species usually found associated with it by the resting position assumed in the water. Violent vibrations of the body and tail, with the anterior and posterior ends of the tail stem as fixed points, produce erratic dashes lasting 10–15 sec., the tail end preceding the body. These movements are followed by longer periods of resting in which the organism floats furcae upward and bent at an angle of 60–80° just below the water surface. The tail stem is almost vertical in position with the anterior one-third and the body turned sharply to the left at an angle of 45°. Movement under a cover glass is produced by similar vibrations or in an anterior direction by alternate attachment of the anterior organ and the ventral sucker. Form changes under a cover glass are very numerous, a spindle shape commonly being assumed by the body.

The above-described Cercaria bears a close resemblance to *C. chrysenterica* Miller in external appearance and in the position of the caeca and penetration glands. Major differences exist however in the number and arrangement of the parts of the excretory system. Also definite caudal glands are present in *C. chrysenterica* and absent in this species. It bears even a closer resemblance to *C. flexicauda* Cort and Brooks (3) in the shape of the organism while in the resting position. Slight metromorphic differences exist between the two species. The flame cells differ in the tail stems of the two species and *C. wardlei* has three pairs of penetration glands while *C. flexicauda* has only two. The oral tuft of bristles so conspicuous in *C. flexicauda* is absent in the above species. The writer is of the opinion that this larva has not been previously recorded and the name *Cercaria wardlei* is suggested.

Cercaria bajkovi n.sp.

Type host: *Limnaea obrussa* and *Physa gyrina*

Type locality: Clear Lake, Manitoba

Specific diagnosis. A slender-bodied pharyngeal longifurcate distome cercaria measuring about 600μ in living specimens. The body and tail stem are of about equal length but each is considerably shorter than the furcae. The body and tail stem are in a straight vertical line and the furcae diverge at an angle of 45–60° when the organism is resting suspended in the water. Two pairs of penetration glands are present anterior to the ventral sucker, which is situated one-fourth of the body length from the posterior end. The esophagus branches into two blind caeca which end in the posterior body region. The anterior organ and ventral sucker bear numerous spines but an oral tuft of bristles is absent and the body is smooth. Four pairs of flame

cells are present in the body, disposed in each lateral half, into an anterior and a posterior group of two. One pair of flame cells is present in the tail stem, and branches of the excretory tube open posteriorly in the midfurcal region.

The average dimensions of moderately extended living specimens under a cover glass are:—body, 189 by 45μ; tail stem, 180 by 32μ; furcae, 228μ. Those of well extended fixed specimens are:—body, 158 by 28μ; tail stem, 135 by 27μ; furcae, 144μ. The anterior organ is about one-fourth as long as the body and is very protrusible and spindle-shaped, the posterior end being sharply pointed. The ventral sucker is circular, about 21μ in diameter and situated about one-quarter of the body length from the posterior end. It is only slightly protrusible. Oral cap and ventral sucker bear numerous retrorse spines. The circumoral spines are long and during the extensive protrusion and retraction of the anterior organ point first forward and then backward. They evidently are of considerable importance in penetration. The tail stem is terminally attached and its surface is finely annulated. About six pairs of caudal glands are present with what appear to be fine ducts leading to the exterior.

The mouth opening is subterminal; a pharynx is present around the narrow esophagus which bifurcates in the mid-body region into two club-shaped caeca. These caeca pass to a position posterior to the ventral sucker. Two pairs of granular penetration glands are located in the body lateral to the esophagus and anterior to the ventral sucker. Their ducts pass anteriorly and open lateral and dorsal to the mouth opening. Four pairs of flame cells are present in the body, disposed in each lateral half into an anterior and a posterior group of two. One pair of flame cells is found in the tail stem, the left being farther posterior than the right. Branches of the caudal excretory tube open posteriorly in the midfurcal region. Striated muscle is present in the tail stem in the form of two heavy lateral bands running from end to end.

The parthenitae are long, branched, motile sporocysts containing a large number of developing cercariae. The diameter is fairly uniform and of about 126μ. The terminations are sharply pointed and the birth pore is subterminal without lips.

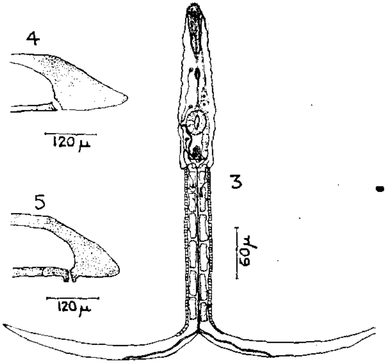

FIG. 3. *Cercaria bajkovi showing internal structure.* FIG. 4. *Cercaria bajkovi, sporocyst showing birth pore.* FIG. 5. *Cercaria elvae, sporocyst showing birth pore.*

Locomotion by this organism in the water is brought about by violent vibrations of the body and tail, the direction of motion being posterior. These periodic dashes are followed by longer resting periods during which the body and tail stem hang exactly perpendicular in the water and the furcae diverge at an angle of 45–60° just below the surface. Locomotion under a cover glass is produced in a similar manner, but creeping in an anterior direction is very limited owing to the inability of the anterior organ to become attached to a flat surface. Great extension of the body is possible and is followed by a flowing of the organs to the anterior end and a drawing up of the posterior body region and tail. During extension the sides of the body are almost parallel and annulated in the anterior half. The anterior end is pointed and the posterior somewhat concave.

C. tenuis Miller bears a greater resemblance to the above-described cercaria than any other recorded species. The distinction between the two is based on the difference in host specificity and marked differences in the arrangement of the excretory organs. The tuft of oral bristles so prominent in *C. tenuis* is absent in the above. These, in the opinion of the writer are sufficient grounds to warrant the creation of a new species for which the name *Cercaria bajkovi* is suggested.

Cercaria elvae Miller 1923

Host. *Limnaea obrussa*

Locality. Clear Lake, Manitoba

A brevifurcate distome cercaria measuring, in living specimens, as follows:—body, 306 by 75μ; tail stem, 360 by 39μ; furcae, 270μ. The spindle-shaped body tapers more gradually from the ventral sucker toward the anterior end than toward the posterior. The ventral sucker is very protrusible and bears two sets of muscle fibres. The anterior organ is large, being 63μ in length. The alimentary canal is a slender tube which bifurcates into two short caeca just anterior to the ventral sucker. Two prominent eyespots are present on the dorsal side and are directly connected with a bilobed mass of nerve tissue which lies just ventral to them.

Five pairs of penetration glands are present and are arranged in two groups, there being two pairs of circumacetabular glands and three pairs posterior to the ventral sucker. The ducts of these glands are prominent and open anterior and lateral to the mouth opening which is subterminal and ventral.

Seven pairs of flame cells are present in the excretory system. Three are anterior to the ventral sucker and empty into the anterior collecting tubule, while three are posterior and empty into the posterior collecting tubule. A seventh pair is present in the proximal region of the tail. The bifurcations of the caudal excretory tubule open at the tips of the furcae.

The parthinetae have not to the writer's knowledge been recorded but were found to be in the form of much branched sporocysts each having a uniform diameter of about 132μ. Each contains large numbers of developing cercariae. The terminations are sharply pointed; the birth pore is subterminal, closely resembling that of *C. bajkovi* but with prominent lips.

The body measurements of this organism are slightly less than those given for *C. elvae* Miller. However the relative body measurements and the arrangement of the body organs are identical. The shape and location of the eye-spots and the number and position of the flame cells indicate that the above organism may correctly be considered as *C. elvae*.

Clinical Notes and Pathogenicity

The greatest number of infestations are found among people who bathe in water six feet or less in depth, as the parasites and their mollusc hosts are almost entirely confined to this region. The organisms are much more plentiful in the water during or directly after dull rough weather, since their liberation is much more rapid in the absence of bright light. About one person out of six appears to be immune to this disease. Women and children are more susceptible than men, although the disease is quite common among men. The majority are reinfected each time they enter the water, since an immunity, if ever acquired, requires considerable time.

Almost immediately upon leaving the water a tickling sensation of the skin is experienced in the infected areas. This continues and gradually develops into a severe itching in about four hours. Pronounced urticaria-like papules with whitish centres and highly inflamed margins appear 12 hr. after infection (Plate I, Fig. 1). Infection is usually much heavier on the arms and legs, as the bathing garments afford some protection to the rest of the body. The papules may be found in groups, or irregularly dispersed in varying numbers. The heaviest infestation observed consisted of 64 papules on the lower limbs of a three-year-old child. The dimensions of the papules vary with the individual from 5 to 15 mm. in diameter and from 1 to 4 mm. high. A hair follicle is usually present in the central elevation.

After 24 hr. the papules are highly inflamed and of a uniform reddish color. The itching is severe. Pus is never present unless secondary bacterial infection is produced by scratching, but in 36 hr. edema is evident and a drop of straw-colored exudate may be squeezed from each papule. Itching and inflammation usually disappear in five days with the regeneration of tissue beginning and the formation of a crust at the site of the papule. In the majority of cases only red macules remain at the end of three weeks.

The penetration of the skin by *C. wardlei* was observed on several occasions. A drop of water containing numerous organisms was placed on the writer's forearm under a binocular. The organisms darted actively about for a time until one of the suckers became attached to a hair. By grasping the hair with the ventral sucker and anterior organ alternately, the organism reached the surface of the skin. The anterior organ was then inserted into the cavity of the hair follicle and by a creeping movement of the body and lashing of the tail, penetration was rapidly effected. When the posterior end of the body reached the surface of the skin the tail broke off and floated away.

The method of locomotion after the skin has been penetrated is not known but an analogous phenomenon has been observed when the organisms were closely confined under a cover glass. Under this condition the anterior end sways slowly from side to side and repeated flowing of the body fluid and organs to the anterior end, with a drawing up of the posterior end, results in an amoeboid-like movement. The penetration glands are active at this time and a copious secretion of colloid-like substance is discharged from the ducts.

By means of this secretion the organism is able to cytolyze the tissue and migrate from the cavity of the hair follicle to the underlying tela subcutanea. Deeper in the subcutaneous tissue a large area surrounding the organism is broken down and an accumulation of fluid swells the space left by the cytolyzed cells (Plate I, Fig. 3). Dissolving of the intercellular substance appears to take place for a considerable distance around the path of the organism with a loosening of the cells and fibres. Large numbers of leucocytes are to be found in masses in the tissue (Plate I, Fig. 4). Whether the organism eventually penetrates a blood vessel and is carried away to remain indefinitely as a hemophilic parasite, or is killed by the resistant forces of the body, is purely a matter of speculation.

All three organisms are capable of causing dermatitis and typical infestations were produced in each case by the author by simply immersing the forearm in water containing a single species of cercaria (Plate I, Fig. 2). Penetration by the organisms is more rapid when the arm is repeatedly dipped into the water and allowed to become almost dry before being returned, than when it is held submerged for the same length of time. Fifteen minutes is a sufficient time of exposure to produce an infection. It appears that the surface tension of the water film assists the organisms in gaining an entrance.

Acknowledgments

This investigation was carried out under the direction of Professor R. A. Wardle, Department of Zoology, University of Manitoba, to whom the writer wishes to express his sincere thanks for much valuable assistance and for the loan of apparatus. Thanks are also due Professor Ferris Neave and Dr. A. D. Bajkov, University of Manitoba, and Mr. C. S. Cunningham, Commissioner of Game and Fisheries, Province of Manitoba. Excision of the tissue shown in Figs. 3 and 4, Plate I, was possible through the courtesy of Dr. Geo. Bedford and Dr. C. E. Corrigan.

PLATE I

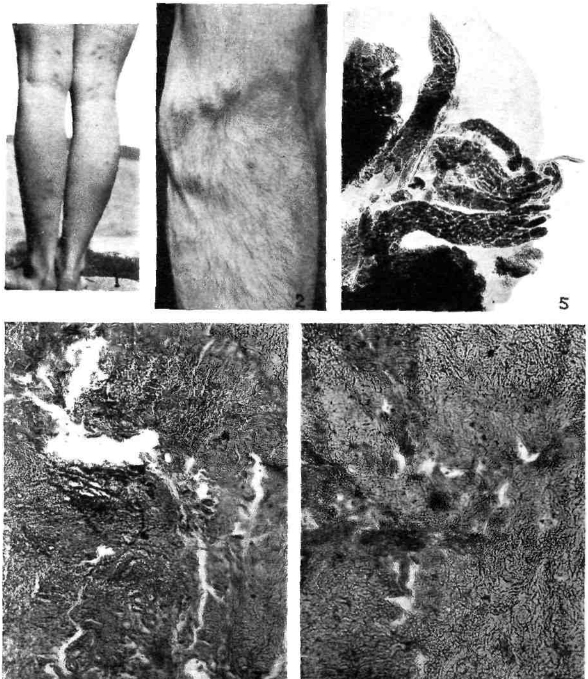

FIG. 1. *Typical infestation on the legs of a bather 12 hr. after exposure.* FIG. 2. *Papules on the writer's forearm 28 hr. after experimental infection with C. wardlei.* FIG. 3. *Photomicrograph of section of typical papule excised from writer's arm, showing path of migration of C. wardlei and loosening of the tissue elements.* ×50. FIG. 4. *Photomicrograph of section of same papule showing edema with masses of leucocytes.* ×50. FIG. 5. *Cercaria bajkovi. Photomicrograph showing sporocyst teased from digestive gland of Limnaea obrussa.* ×50.

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FLUKE DISEASE IN NORTHERN MANITOBA SLEDGE DOGS[1]

By J. A. Allen[2] and R. A. Wardle[3]

Abstract

An opisthorchiid Trematode, *Parametorchis manitobensis* n.sp. is recorded from the livers of sledge dogs from northern Manitoba. A key is given to the known species of *Parametorchis*. Infection with the trematode produces chronic cirrhosis and anemia, followed by muscular weakness and death. Infection is believed to be contracted by ingestion of metacercariae in *Catostomus commersonii* and prophylaxis would consist of the thorough boiling of such fish before use as dog food.

Introduction

While serving as a member of the expeditionary party, formed by Dr. R. G. Green in the summer of 1933 to study pathological conditions in grouse and wild rabbits of northern Manitoba, one of the writers (Allen) discovered the presence of numerous liver flukes in sledge dogs. The trapper who submitted the carcasses for autopsy, reported that nearly all his young dogs had died from an obscure disease and that he and other trappers in the vicinity of Mile 42 on the Hudson's Bay Railway (Cormorant Lake) were finding it increasingly difficult to raise dogs.

The existence of such insidious parasitic infestation in the dogs of these isolated regions is of great significance to trappers and others who are dependent upon sledge transportation during many months of the year. Dogs north of the 53rd parallel serve the inhabitants very much as horses did in settled districts 40 years ago, and a well conditioned team of huskies often takes the wayfarer through many a hazard.

In recent years a number of reports concerning heavy mortality in sledge dogs have come out of the North Country. Some of these reports and descriptions would indicate that an infectious, distemper-like disease was responsible for the mortality, but the lay mind is prone to confusion in matters pertaining to disease and pathology, and some of the outbreaks, perhaps, may be attributable to fluke disease and other forms of parasitism.

Further research in northern territories, where raw fish is the common diet of canines, will probably reveal that liver fluke disease is quite rampant. It is interesting to note here that Dr. J. K. Morrow, a practicing veterinarian of Winnipeg, found a fairly heavy liver fluke infestation in the cadaver of a husky dog brought to him for post-mortem examination on January 9, 1934. This subject was a member of a team of dogs which had travelled from the town of The Pas, located just 42 miles south of the district in which the writers' first cases were observed. Furthermore, this trematode proved to be of the same species as that found in the writers' cases. Fecal tests were

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made by the flotation and centrifugal methods on the seven remaining dogs of this team, and five of them were found to be lightly involved, only two or three ova being found in each smear.

Symptomatology

All the cases observed in the writers' brief clinical study had reached a stage of chronicity and consequently the manifestations of early infection could not be recorded. It is inconceivable, however, that there could be heavy invasion by a devastating parasite like the liver fluke without concomitant systemic reaction in the early stages. However, as is the case in most forms of internal parasitism, light infections may go unnoticed until the fecal test is applied. There is always the quantitative factor.

Progressive weakness is characteristic of the malady. The affected animal tires easily and lags in the traces; frequently it quits on the trail and has to be carried on the sledge to the next stopping post. In advanced stages, cachexia and marked emaciation are prominent (Fig. 1). When turned loose, the animal seeks seclusion and sometimes becomes lost in the bush. Often it wades into water and stands there in a stupor for a protracted period. Icterus was seen in one case, but it does not appear to be an outstanding feature of the disease. Prior to this investigation, the owner had noted nervous symptoms, simulating those seen in encephalitis, in a few of the diseased dogs.

Symptomatic anemia was very pronounced in all the cases coming under observation. Ascites, which appears to be a character of liver fluke disease in cats, was not evident in the cases under review.

Before death, the patient is so exhausted that it cannot rise without assistance, although the eyes may appear fairly bright and intelligent-looking. When raised to its feet, the animal stands as though propped on four sticks instead of articulating limbs (Fig. 1). The patient may remain in this condition for four or five days before death which is signallized by deep coma.

Some of the cases were complicated by a fairly heavy infestation with immature individuals of a species of *Diphyllobothrium*, probably *cordatum*, and it is difficult to say which part of the foregoing syndrome was referable to their presence.

Etiology

Description of the Parasite

The causative organism is a Trematode of the family Ophisthorchiidae Braun and the genus *Parametorchis* Skrjabin.

Four species of this genus have been previously recorded, namely:—*complexus*, originally described by Stiles and Hassall (4) from cats in New York, Maryland and District of Columbia, and more recently described by Essex and Bollman (1) from a cat at Rochester, Minnesota; *noveboracensis*, described by Hung (2) from a cat at Ithaca, New York; *intermedius*, described

by Price (3) from a specimen of *Vulpes fulva* in Wisconsin; and *canadensis*, described also by Price from a specimen of *Putorius vison* from Kirkfield, Ontario.

The present material shows a combination of characters differing from those of the four recorded species and in the writers' opinion it represents a hitherto undescribed species for which the name *manitobensis* is suggested.

From several hundred available specimens, 20 uninjured individuals were selected at random upon which to base the following specific description. The external dimensions are based upon 10 specimens preserved in 5% formalin; the internal dimensions are based upon the remaining 10 specimens, lightly stained with Grenachers borax carmine, cleared in beechwood creosote and mounted in Canada balsam.

Parametorchis manitobensis n.sp.

Specific diagnosis.—Parametorchis: body typically pyriform, although many individuals are linguiform, tapering cephalad, rounded caudad, translucent, minutely spinose; 5.5–6.6 mm. long by 1.7–2.6 mm. wide, greatest width between the testes or at level of posterior testis; oral sucker terminal, weakly muscular, wider than long, 25–40 μ long by 33–42 μ wide; acetabulum conspicuous, situated on low papilla, one-quarter to one-third of the body length from the oral sucker, weakly muscular, circular, 32–40 μ in diameter.

Prepharynx absent; pharynx strongly muscular, longer than wide, 25–35 μ long by 22–30 μ wide; esophagus very short; intestinal caeca sinuous, 18–25 μ wide, ending 20–25 μ from posterior body edge.

Genital pore median, at junction of first and second fifths of body length; ovary mediolateral, at middle of body length, oval to pyriform, long axis oblique and 60–80 μ in length; vitellaria in two lateral bands, extending cephalad from level of ovary to level of point midway between acetabulum and caecal fork, not united in front of uterus; uterus wholly in anterior body half, post-acetabular portion with transverse coils, pre-acetabular portion rosettiform; eggs 26–15 μ in dimensions; testes arranged tandem, wholly in posterior body half, markedly lobulate; anterior testis with three to five lobes, 50–70 μ long by 80–90 μ wide; posterior testis with five to nine lobes, 60–80 μ long by 65–85 μ wide.

Excretory stem sigmoid, between testes, nearly or as wide as caeca.

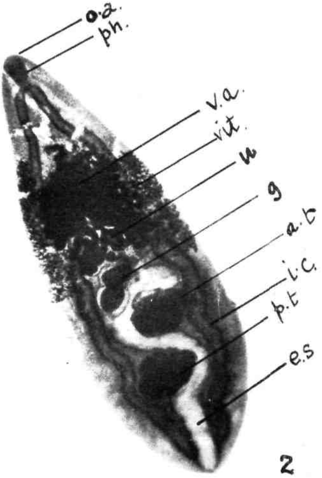
Host. *Canis familiaris.*

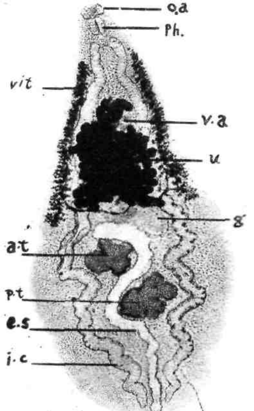
Location. Hepatic ducts.

Distribution. The Pas district, northern Manitoba.

Location of type of material. U.S.N. Museum.

From *complexus* this species is distinguishable by the absence of any preuterine commissure of the vitellaria; from *intermedius*, it is distinguished by its larger size, by the partly rosettiform uterus, by the wide excretory stem; from *noveboracensis* and *canadensis*, it is distinguished by the lobulated testes and the sinuous intestinal caeca (Figs. 2 and 3).





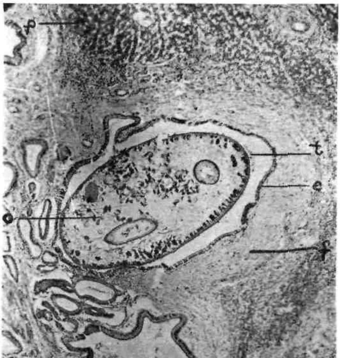


FIG. 1. *An advanced case of liver fluke disease.* FIG. 2. *Parametorchis manitobensis* × *16; o.a., oral sucker; p.h., pharynx; v.a., ventral acetabulum; u., uterus; vit., vitellaria; g., ovary; a.t., anterior testis; p.t., posterior testis; e.s., excretory stem; i.c., intestinal caecum. (Photographed without filter.)* FIG. 3. *As in Fig. 2; photographed through Wratten M (G-filter).* FIG. 4. *Section of liver* × *40; t., trematode in situ; o., ova of trematode; e., epithelium of bile duct; f., fibrosed wall of bile duct; p., cells of hepatic parenchyma.*

The following key to the known species of *Parametorchis* illustrates these differences:—

a_1. Testes markedly lobulate; intestinal caeca sinuous.

b_1. vitellaria uniting in front of uterus; body 5–7 mm. long.......................................*complexus.*

b_2. vitellaria not uniting in front of uterus;

c_1. uterus rosettiform, excretory stem wide, body 5–7 mm. long.................................*manitobensis.*

c_2. uterus in transverse coils; excretory stem narrow; body 3–3.5 mm. long.........................*intermedius.*

a_2. Testes smooth or only slightly lobulate; intestinal caeca straight.

d_1. uterus rosettiform; testes almost round; body length 6–6.3 mm...........................*noveboracensis.*

d_2. uterus transversely coiled; testes oval or slightly lobed; body length 1.7–2.0 mm.................*canadensis.*

It may be noted that no opisthorchiid trematode has previously been recorded from the dog in North America, but *Amphimerus pseudofelineus* (Ward) was originally described from *Canis latrans* in Nebraska.

Pathology (Gross)

The outstanding pathological change is a hypertrophic cirrhosis of the liver, which is increased to two or three times the normal size and weight. In the field the writers were not equipped to weigh the specimens, but the body weight of the dog examined by Morrow was 56 lb. (25.40 kgm.) and the liver weighed 3 lb. 14 oz. (1.75 kgm.).

The liver is hard to the touch; its capsule yellowish in color, and its surface exhibits a varying number of hard, yellowish and circular elevations. The edges of the liver are markedly rounded.

When the organ is incised, a brownish green, fluke laden material oozes from greatly dilated bile ducts, some of which are as large in diameter as an ordinary lead pencil. In one of the fresh specimens the ducts were completely occluded by fluke laden exudate which appeared in motion when viewed through a binocular giving a magnification of eight diameters.

The wall of the gall bladder is thickened, and the bladder is engorged with dirty, greenish inspissated bile which teems with flukes and their ova.

Pathology (Microscopic)

Because of the enormous number of parasites in the specimens, no difficulty was experienced in obtaining microscope sections of liver showing the fluke, with its ova, *in situ* (Fig. 4).

In the few cases studied, the cells of the hepatic parenchyma were essentially normal, but demonstrable changes were evident in the bile ducts and interlobular connective tissue. The former had thickened fibrosed walls, sur-

rounded by areas of less-dense connective tissue. The interlobular connective tissue appeared to have undergone a simple hypertrophy. There was little or no evidence of congestion.

Transmission

Undoubtedly the direct vehicle of infection is raw lake fish. The severely affected dogs at Cormorant Lake were fed during the spring and summer months entirely on suckers (*Catostomus commersonii*), but the dogs from The Pas received in addition white fish (*Coregonus clupeaformis*), pickerel (*Lucioperca vitreum*), and lake trout (*Cristivomer namaycush*). The difference in diet may explain the difference in degree of infestation noted in these separate groups of dogs.

Since circumstantial evidence pointed to the sucker as the chief host of the metacercariae, attention was first directed to this fish. During the present winter 60 suckers were obtained from Cormorant Lake, but the writers have been unable to demonstrate the presence of the intermediate form in any of them. While extending the preliminary survey to include other kinds of lake fish, the writers are keeping in mind the possibility that suckers serve only as seasonal hosts of the metacercariae. Such seasonal occurrence would break the continuity of fluke infection in dogs, and give some time for establishing the relative resistance which seemingly exists against the disease.

Prophylaxis

Two groups of trappers' dogs, kept in runways within a few hundred yards from the affected animals at Cormorant Lake, were clinically free from disorder. One of these groups was fed on cooked fish, the other group exclusively on boiled cereals. The trapper who boiled the fish before feeding it to the dogs informed the writers that he and other dog breeders in the vicinity had experienced much difficulty in raising dogs prior to the adoption of this practice. He conveyed the idea that raw fish was falling into disrepute as a diet for sledge dogs, although the true cause was not suspected.

Acknowledgments

The writers' thanks are due to Mr. A. G. Cunningham, Director, Manitoba Game and Fisheries Branch, for permission to publish this report; to Dean Alfred Savage, University of Manitoba, for advice during the preparation of this report; to Mr. J. M. Isa for preparing some excellent tissue sections.

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OBSERVATIONS ON THE VARIABILITY OF *POLYSPORA LINI* LAFFERTY[1]

By A. W. Henry[2]

Abstract

Polyspora lini, the fungus which causes the browning and stem-break disease of flax, was isolated from flax material obtained from Ireland, Sweden, the United States and Canada. Cultural differences in pigmentation and growth characters were exhibited between different strains when grown on potato dextrose, corn meal and prune agars.

Saltants arose in the form of sectors from most of the parent cultures. The parent strain from Ireland was particularly prolific in the production of saltants. New strains differing from parental strains and others in cultural characters originated in this way. Many of these in turn produced sectors. Monosporous strains appeared to produce saltations as readily as polysporous strains.

Most of the original isolations yielded a special form of the fungus, characterized by the production on all media tested of firm, tough colonies consisting of abundant densely packed mycelia and a relatively sparse development of spores. On the same media the usual form of the fungus develops soft, loose, profusely sporulating colonies. Strains of the new form of the fungus have arisen as saltants either as sectors or as islands in colonies of the ordinary form of the fungus. They have thus far failed to revert to the parental type even after long culture on artificial media or on passage through the host plant.

Preliminary pathogenicity tests indicate that the same variety of flax may differ in its reaction to different strains of *Polyspora lini*, so that strains of this fungus may differ both in cultural characters and in pathogenicity.

Introduction

Cultures of *Polyspora lini* Lafferty, the fungus causing the browning and stem-break disease of flax have been under the observation of the writer for a period of 10 years. These cultures have been derived from diseased flax obtained from widely separated parts of the world and hence should be suitable for the detection of any variability which may exist in the fungus.

Previous Work

Relatively little has appeared in the literature concerning *Polyspora lini* since its original description by Lafferty (2) in 1921. His paper consequently still provides the principal information which we have on the morphology and cultural characteristics of the fungus and also on the disease which it causes. In respect to the constancy of its cultural characters he concludes that "irrespective of its source of origin, the fungus behaved similarly on the various media and showed no morphological or cultural differentiation into strains or races."

Sources and Isolation of the Fungus

Polyspora lini was first isolated by the writer in 1923 from diseased flax stems collected at Saskatoon in that year. It was again isolated in 1924 from

[1] *Manuscript received January 24, 1934.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Alberta.
[2] *Associate Professor of Plant Pathology, Department of Field Crops, University of Alberta.*

flax leaves collected at Saskatoon in 1920 (1). In 1925 it was isolated from diseased flax stems obtained from the United States, Sweden and Ireland. The material from Ireland was of special value for comparative purposes as it was kindly supplied by Mr. H. A. Lafferty, the author of the original description of the fungus. Later in 1930 isolations were made from collections obtained from two points in the province of Alberta, Canada.* The sources of the various cultures and the collectors of the diseased material are indicated in Table I. The pathogenicity of most of the above collections has been proved.

TABLE I

SOURCES OF CULTURES OF *Polyspora lini* UNDER OBSERVATION

Culture	Country	Town	Part of flax plant cultured	Collector
A	Canada	Saskatoon, Sask.	Leaves	Henry
B	Sweden	Svalof	Stems	Sylven
C	Ireland	Dublin†	Stems	Lafferty
D	United States	East Lansing, Mich.	Stems	Robinson
E	United States	St. Paul, Minn.	Stems	Henry
F	Canada	Edmonton, Alberta	Stems	Henry
G	Canada	Provost, Alberta	Seed	Fullerton

†*Received from Dublin.*

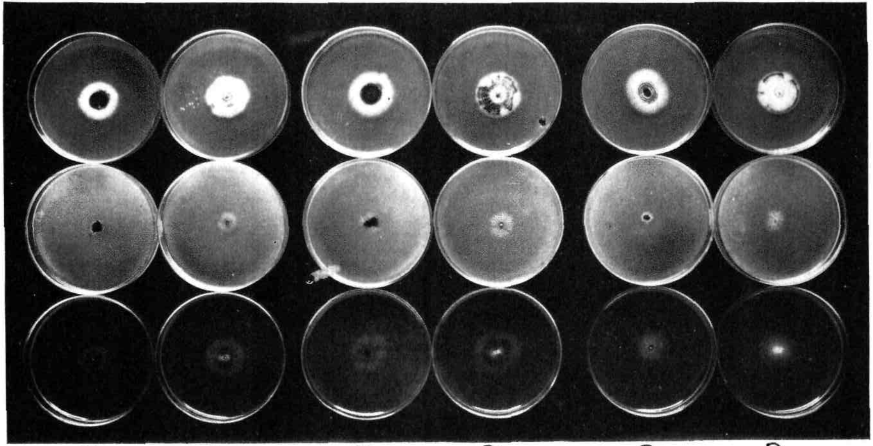
The fungus was readily isolated after surface sterilizing bits of diseased tissue with mercuric chloride (1–1000) and plating them on potato dextrose agar. Profusely sporulating colonies typical of *Polyspora lini* commonly arose in a few days from such tissues. These usually were light in color at first, Plate II, Fig. 2, but soon t rned dark owing to the production of a black pigment, Plate II, Fig. 3. In the case of the Swedish material, cultures were obtained by placing the diseased stems in a moist chamber to induce sporulation, suspending the spores in tubes of potato dextrose agar and sowing plates with the spore suspensions. Monosporous cultures were later made from most of the original isolations and from many of the variants which arose.

Media Employed

Since the fungus grew well on freshly prepared potato dextrose agar this medium was used for ordinary cultural work and for the preservation of the different isolations. In addition three commercial agars, namely Bacto potato dextrose agar (pH 5.5), Bacto corn meal agar (pH 6.1) and Bacto prune agar (pH 5.8) were used for comparative studies. As indicated below, these media proved effective in bringing out cultural differences between different isolations of the fungus. Had the same media been employed by Lafferty he undoubtedly would have obtained similar results. In his investi-

**The assistance of two graduate students, Mr. E. G. Sharvelle and Mr. J. B. Skaptason, in isolating and photographing cultures of these Canadian collections is gratefully acknowledged.*

PLATE I



Comparison of cultural characters of six strains of Polyspora lini on three kinds of agar; namely, potato dextrose (left), corn meal (centre) and prune (right) after 11 days at room temperature. Each row of three plates represents a separate strain. These strains were derived from the following sources: C, from Dublin, Ireland; A, from Saskatoon, Saskatchewan, Canada; D, from East Lansing, Michigan, United States; D_1, from a sector of D; B, from Svalof, Sweden; and B_1, from a sector of B.

gations the fungus was grown on ground Quaker oat agar, beef extract agar, cooked flax extract agar and on beef extract, extract of Quaker oats, gelatin and cooked green flax stems. As already noted, the fungus behaved similarly on these media. It developed its mycelium comparatively sparsely and slowly in all cases, but always produced its conidia in great abundance.

Nature and Origin of Variability

In comparing the colony characters of cultures of *Polyspora lini* from different sources it was noticed that, while the majority resembled one another rather closely, there were some that differed quite widely from the rest. This is shown in Plate I by monosporous strains of the fungus from Ireland *C*, Canada *A*, the United States *D*, and Sweden *B*, growing on three different kinds of agar under similar conditions at room temperature. It will be noted that the strains from Ireland *C*, and the United States *D*, resemble each other closely on each of the three different media, whereas those from Sweden *B* and from Canada *A* differ considerably from *C* and *D* and also from each other. The most conspicuous difference is in the amount and distribution of the black pigment in the colonies. The Canadian strain *A* on potato dextrose and on corn meal agar remained relatively free from this pigment up to the time the photographs were taken, while the Swedish strain *B* produced an intermediate amount.

All the original strains *A* to *G* tended to produce sectors under certain conditions regardless of whether the parent cultures were derived from single spores or not. The strain from Ireland *C* was particularly prolific in the production of sectors, as is shown in Plate II, Fig. 4. Sub-cultures, both mass and monosporous, derived from these sectors commonly differed from their parents in pigmentation, habit of growth and other characteristics. In Plate I monosporous strains D_1 and B_1 obtained from sectors of *D* and *B* are compared with their parents on three kinds of agar. It will be noted that each differs from its parent on all three media. On prune agar B_1 shows particularly striking differences in habit of growth from its parent and also from all the other strains illustrated in Plate I.

Large numbers of saltants arising in the form of sectors have been observed, but only relatively few have been isolated. Saltation appears, however, to be the principal method of origin of new strains. In general, such strains exhibit as great a degree of constancy as their parents, producing a relatively uniform type of colony on a given medium. This is shown in Plate II, Fig. 1, in which B_1, a monosporous strain derived from a sector of *B*, is shown growing on triplicate plates of each of three different kinds of agar. The colonies as will be noted are quite uniform on the same kind of agar. However, on potato dextrose agar a tendency toward further variation is shown in the formation of sectors from which further strains might be derived.

Occurrence of Firm Sparsely Sporulating Strains

The usual type of colony of *Polyspora lini* described by Lafferty and observed by the writer is a flat, soft, profusely sporulating one, with a com-

paratively poorly developed mycelium. Such colonies are greasy in appearance and turn black with age. It was of interest, therefore, to encounter during the course of these studies a form of the fungus quite different from that just described. This contrasts with the ordinary form in producing colonies with relatively few spores but with vigorous well developed mycelia. The colonies may be described as firm, tough or leathery and are often more or less raised and furrowed. They are not greasy in appearance and vary in color from light to dark gray. Spores are produced, but in greatly reduced numbers compared with the usual form of the fungus. In fact the name *Polyspora* hardly seems appropriate for the new form. It would seem that the ability to produce this form is inherent in *Polyspora lini* generally, since in these studies it has been obtained from normal isolates of the fungus obtained from flax collections made in Ireland, the United States and Canada.

The new form of the fungus so far has shown greater constancy than the normal form on various media. Up to the present it has not reverted to the normal parental type. Some strains have retained their characteristics in culture for seven years. Even on passage through the host plant it has retained its characteristics. It has not as yet been derived directly from the host plant except following artificial inoculation with the same form of the fungus.

The strains of the new form so far observed have arisen from normal colonies only and in either of two ways: 1, in the form of sectors; and 2, in the form of islands (Plate II, Fig. 5). There has been no consistent difference observed between strains derived in the first way from those derived in the second way. In fact the origin may be the same in both cases, the only difference being in habit of growth afterwards. Though the new form of the fungus exhibits greater constancy than the normal form, different strains are not all alike on cultural media. There are differences in color of colony and perhaps in other characters between different strains of the new form.

Variation in Pathogenicity

Artificial inoculation studies have indicated that some varieties of cultivated flax, *Linum usitatissimum*, are more susceptible to *Polyspora lini* than others. The variety Bombay C.I. 42 and certain other Indian selections have proved among the most susceptible tested, whereas Winona, Ottawa 770B and others have shown considerable resistance. However, different strains of the fungus do not necessarily produce the same reaction on the same variety of flax. Thus one of the Indian selections, Type 12, developed distinct lesions when inoculated with strain *A* but none when inoculated with *C*. Bombay also developed distinct stem lesions when inoculated with strain *A* but none when inoculated with a hard (new type) saltant of *A*. This does not necessarily mean that all strains of the new type are of reduced pathogenicity compared with their parents. Further work will be necessary before this can be decided. In the case of saltants of the normal type evidence has been obtained that some show a reduction in pathogenicity while others exceed

their parents in this respect. The work done thus far on the host relations of the fungus then, while only of a preliminary nature, indicates that varieties of cultivated flax differ in their reaction to ***Polyspora lini***, and that this fungus exhibits specialization in pathogenicity as well as in cultural characters.

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EFFECT OF FROST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

I. PHYSICAL CHARACTERISTICS OF THE KERNELS[1]

BY R. NEWTON[2] AND A. G. MCCALLA[3]

Abstract

Samples of freshly cut wheat were exposed in freezing chambers, then stook-cured, threshed, and compared with unfrozen check samples, in the seasons 1929, 1930 and 1932, at Edmonton, Alberta. The check samples attained approximately maximum grade and weight per bushel when cut at a stage of maturity represented by a dry matter content ranging in different varieties and seasons from 50 to 60%. The weight per 1000 kernels of Marquis wheat, determined in 1930 only, reached a maximum at the same stage as weight per bushel. Susceptibility to superficial injury by frost continued generally over nearly the whole maturation period. Even 4 degrees of frost (28° F.) frequently caused a cut in grade, while 8, 10 and 14 degrees caused more substantial degradation, often accompanied by some reduction in weight per bushel. Marquis wheat is apparently more susceptible than Garnet, Reward and Red Bobs, to degradation by frost during the maturation period. Grade, being based on external appearance, is more sensitive to frost than are chemical composition and baking quality. Classification of the kernels of Marquis, in 1930, into vitreous, starchy, immature, green, bran frosted and heavily frosted, confirmed the earlier conclusion (2) that only the sound class (vitreous + starchy) has enough relation to grade to be useful as a grading factor. The germination of Garnet was improved by moderate freezing.

Introduction

Every year a certain proportion of western Canada's wheat crop is frozen before reaching maturity, and the effect of such freezing on the physical characteristics, chemical composition, and milling and baking quality of the grain is of great practical importance in relation to the problem of equitable grading and merchandising. In the fall of 1928 an unusually large proportion, in fact over half, of the crop was damaged by frost. The grades were low, the yields reduced in some cases, and the financial loss to the farmers exceedingly heavy. In view of this serious situation, the Associate Committee on Grain Research of the National Research Council undertook a study of various aspects of the problem.

The problem was attacked in two ways. First, a survey was made of the crop of 1928, the milling and baking quality of a large number of commercial samples being studied in relation to the grades given them as a result of frost injury. A report on this has been published by Geddes, Malloch and Larmour (2), and further surveys have been carried on annually, the results of which will be published in due course.

[1] *Manuscript received January 30, 1934.*
Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. Acknowledgment is made to the officers of the Western Grain Inspection Division, who graded all the samples, and of the laboratory of the Dominion Seed Branch at Calgary, who carried out the germination tests. Issued as Paper No. 49 of the Associate Committee on Grain Research.
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[3] *Research Assistant, Associate Committee on Grain Research, University of Alberta, Edmonton.*

The second method of attack was to produce artificially frosted grain of known history under controlled conditions. A freezing plant was installed at the University of Alberta, with which there was produced in 1929, 1930 and 1932 three series of samples subjected to graded frost exposures at progressive stages of maturity. These samples have provided the material for studies of physical characteristics, chemical composition, milling and baking quality, and seed value. This series of papers deals with the results obtained under the first three headings. The study of seed value has been carried out in co-operation with the Calgary laboratory of the Dominion Seed Branch, and will be reported later.

The work as a whole is adding substantially to the existing body of knowledge in regard to the properties and value of frosted wheat, and it is hoped that it may be of practical assistance in the consideration of grading problems.

Production of Material

In 1929, four varieties of wheat—Garnet, Reward, Marquis and Renfrew—were grown on clean fallow land (near Edmonton), and collections were made at progressive stages of maturity of the wheat, artificially frozen samples being produced from each. Cutting was started when the wheat kernels contained about 40% dry matter, it being judged, in the absence of experience on the point, that this was probably the earliest stage from which a milling sample could be secured. Sampling was continued until the wheat was ripe. The plots were long and narrow, and each cutting consisted of one swath the entire length of the plot.

About 45 sheaves of each of two varieties were cut as early in the morning as conditions would allow (usually between 8 and 9 o'clock). These were labelled in rotation as they fell from the binder, *A*, *B*, and Check, and directly afterwards brought by motor truck to the freezing plant. This plant had two chambers, which made it possible to freeze two lots at different temperatures each day. Each chamber contained two racks of chicken-wire shelves 6 in. apart, with a capacity of 10 sheaves of wheat cut open and spread out not more than 2 in. deep. The *A* and *B* sheaves were spread on the shelves in the inner and outer chambers respectively, and the ice machine started. The air in each chamber was circulated by means of a Sirocco fan connected with flues opening at various levels. An attempt was made to have the temperature curves simulate those of a natural frost. The rooms were cooled to minimum temperatures ranging from 30° to 18° F., these minima being maintained for one to two hours, after which the machine was shut off, and the doors opened.

Since a crew of two men was doing the sampling and freezing, it was necessary for them to remove the grain from the chambers in the evening, in order that the chambers might be free to receive the fresh samples they brought in the following morning. Thus the sheaves had to be retied and removed shortly after the doors were opened, a procedure that involved more rapid

warming up than would probably occur in nature. This was overcome in subsequent seasons by increasing the number of workers.

The *A* and *B* sheaves were stooked outside with the unfrozen check sheaves, and allowed to cure normally, except that all were capped with canvas covers at night and during rain, to prevent weathering. As soon as the grain was sufficiently dry, it was threshed. The samples were cleaned with special precautions to avoid the loss of small, frost-injured kernels, and were used for analytical and milling and baking studies.

In 1930, effort was concentrated on producing a homogeneous series of samples as long and complete as possible, for elaborate investigation. Only one variety, Marquis, was used. This was grown under conditions similar to those of the previous year, and sampled in much the same way, but beginning at an earlier stage, when the kernels contained about 30% dry matter. Moreover, profiting by the previous season's experience, when the frost exposures caused less damage than had been expected, the treatment in this respect was made more rigorous. With minor variations, the chambers were cooled to 28° and 24° F., and to 22° and 18° F., respectively, on alternate days, and the minimum temperature was maintained for four hours. The machine was shut off at the end of this period, but the wheat was left in place and the chambers kept closed till the following morning. The temperature therefore rose gradually, more nearly approaching natural conditions. True, the length of exposure to the minimum temperatures exceeded that usually found in a night frost, but in nature each spike of wheat stands separately and would doubtless cool more rapidly than under the experimental conditions described, notwithstanding the care taken to provide a good circulation of air.

In 1932, with the limited object of comparing the effect of frost on the grade and weight per bushel of a few well known varieties, and of producing seed for the co-operative study with the Dominion Seed Branch, the following varieties were used: Garnet, Reward, Red Bobs, Marquis. Sampling was deferred until the grain was sufficiently developed to be of interest from the practical grading viewpoint. Since smaller samples were adequate, it was possible to accommodate all four varieties in each frost chamber in a given run. The chambers were cooled to 28° and 22° F., respectively, held at the minimum temperature for about four hours, and allowed to warm up gradually.

This paper deals with the physical characteristics of the frozen wheat samples so produced in 1929, 1930 and 1932, in comparison with the corresponding non-frozen check samples. The chemical composition and milling and baking quality of the 1929 and 1930 series will be dealt with in succeeding papers of the series.

Previous Work

While it is not intended to present here a review of the literature on the physical characteristics of frozen wheat kernels, reference will be made to a few papers bearing directly on the points to be dealt with in the following pages.

Johnson and Whitcomb (3) published a valuable summary of information accumulated by themselves and others over a period of 10 years at the Montana Experiment Station, on both physical and chemical properties, and milling and baking qualities, of normal and frosted Marquis wheat. They found that when the kernels contained not more than 44 to 46% moisture, a stage they described as "stiff dough" and corresponding to the attainment of practically full kernel and bushel weight, frost appeared to have no further effect on milling and baking quality. Moisture content did not, however, furnish a clearcut line above or below which frosted kernels were either present or absent. Quite mature wheat showed a high percentage of wrinkled or blistered kernels after exposure and, other things being equal, this percentage largely determined the grade of the wheat. Frosted wheat had a lower weight per bushel than non-frosted wheat of the same stage of maturity. This was not due to a change in true density, but was attributed to the looser packing of the blistered kernels. Exposure to weathering further decreased the weight, an effect ascribed to leaching.

Arny and Sun (1), in experiments at St. Paul, Minnesota, found that both the kernel weight and bushel weight of Marquis wheat increased until within four days of maturity, though the change within a week of maturity was not sufficient to affect the grade. The kernels in the terminal spikelets were in the hard dough stage seven days before maturity, but in the lower spikelets did not attain this stage until two days before maturity. In line with the foregoing, Shutt (4) found that Reward wheat at Beaverlodge, Alberta, in 1928, cut at two-day intervals beginning at the early dough stage, attained the grade of No. 1 Northern and maximum kernel weight at the sixth cutting, though it was not considered ripe for normal harvesting until about two cuttings later. Garnet wheat in the same season at Lacombe, Alberta, cut at similar intervals, improved until it reached No. 2 Northern at the soft to medium dough stage, when it also attained its maximum kernel weight. Successive "slight frosts" beginning at this point, reduced all succeeding grades to Nos. 4, 5, 6 and Feed.

Geddes, Malloch and Larmour (2), as already noted, published the first report on the program of investigations on the grading of frosted wheat, of which the present paper forms a part. Since commercial grading is done on the basis of physical characteristics, these authors directed their enquiry chiefly to the relation between the proportions of such physical types as sound, frost-damaged, and immature kernels in a sample and its milling and baking quality. One chemical test was included, namely, the protein content, and this proved the best single measure of baking quality within each grade, though it was not a reliable measure when comparisons were made between the grades, owing to differences in protein quality. As grading factors for frost-damaged wheat, the only physical characteristics which were sufficiently related to milling and baking quality to promise usefulness were the weight per measured bushel and either the percentage of total sound or of hard red vitreous kernels.

Series of 1929

The grade and weight per measured bushel in relation to the stage o maturity and exposure to frost of the 1929 samples are shown in Table I

TABLE I

GRADE AND WEIGHT PER BUSHEL IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1929

Date of cutting	Dry matter at cutting, %	Degrees of frost, F.		Grade			Weight per bush., lb.		
		A	*B*	*A*	*B*	Check	*A*	*B*	Check
Garnet									
Aug. 1	43.4	7	4	5	4	4	60	60	60
Aug. 3	45.5	5	3	3	3	3	63	63	62.5
Aug. 6	49.0	8	6	3	3	3	64	64	63.5
Aug. 8	49.5	5	3	2	2	2	65	64	64.5
Aug. 12	57.4	4	2	3	2	2	65	64.5	65.5
Aug. 14	59.6	8	6	3	2	2	64.5	65	65.5
Aug. 16	60.6	9.5	8	3	2	2	65.5	65	65.5
Aug. 21	65.0	14.0	9	4	4	2	65	65	65.5
Reward									
Aug. 1	41.5	7	4	6	6	6	59	59.5	58
Aug. 3	42.9	5	3	5	5	5	63.5	63.5	63
Aug. 6	49.0	8	6	5	5	4	64	64	64
Aug. 8	48.2	5	3	3	3	3	65	65	65
Aug. 12	52.6	4	2	3	3	3	66	66	66
Aug. 14	54.3	8	6	3	3	2	66.5	66.5	66
Aug. 16	56.6	9.5	8	4	3	2	66.5	66.5	66
Aug. 21	63.0	14	9	5	5	2	66	66.5	66.5
Marquis									
Aug. 15	46.8	4.5	3	4	4	3	63	63	62.5
Aug. 17	48.8	8	6	3	3	3	64	64	63
Aug. 20	53.7	9	7	4	3	2	65	65	65
Aug. 22	54.9	9.5	8	5	4	2	65	65	65.5
Aug. 23	56.7	9	7	4	4	2	65	65.5	65.5
Aug. 24	57.6	8	6	4	3	2	65.5	65.5	66
Aug. 26	58.8	13	11	5	4	2	65	65.5	66
Aug. 27	59.0	9	7	5	4	2	65.5	65.5	66
Aug. 29	66.6	7	5	3	3	2	66	66.5	66
Renfrew									
Aug. 15	40.5	4.5	3	4	5	4	57	57	57.5
Aug. 17	46.2	8	6	4	4	4	59.5	59.5	59
Aug. 20	50.1	9	7	3	3	3	63	63	62.5
Aug. 22	52.7	9.5	8	5	4	2	63.5	64.0	64
Aug. 23	54.4	9	7	4	4	2	63.5	63.5	64.5
Aug. 24	52.3	8	6	3	3	2	64.5	64.5	64.5
Aug. 26	57.7	13	11	4	4	1	64	64	65
Aug. 27	56.5	9	7	4	3	2	64.5	65	65
Aug. 29	60.5	7	5	2	2	2	65	65	64.5

The stage of maturity is indicated by the dry matter content of the kernels at the time of cutting, it being believed that this is a safer and more precise measure of this property than the descriptive terms ordinarily employed, such as early milk, milk, late milk, early dough, etc., which depend largely on personal judgment. Not only does practical observation support such a belief, but also the study of chemical development in relation to moisture content to be reported in the succeeding paper. In 1929, the varieties were cut in pairs on any given day, the sampling of Garnet and Reward covering the period August 1 to August 21, and that of Marquis and Renfrew the period August 15 to August 29. Thus within each of these pairs the dry matter content at successive samplings may be taken to indicate their relative rates of progress towards maturity during these periods. Such a general relation may of course be interfered with, especially in later stages, by absorption of moisture in rainy weather.

The grade has been found more sensitive to frost than are composition and baking value. Although in 1929 the frost exposures caused less damage than had been expected, the effect of the more severe exposures is clearly seen in the grades. Nor is it confined to the more immature samples but occurs also in the most mature ones, as witness the last cuttings of Garnet and Reward. While these, it is true, suffered particularly severe exposure, it should be noted that 9 degrees of frost caused as much degradation as did 14 degrees. All varieties were judged to be fully ripe, from the standpoint of readiness for binder harvesting, when the last samples were taken, yet there is no evidence in the grades that they had become less sensitive to frost injury up to that stage. Wrinkling and blistering of the bran, in the absence of more deep-seated effects, are sufficient to degrade wheat seriously.

In the check (unfrozen) samples, the grades improved as the varieties progressed towards maturity, until the dry matter content reached about 50 to 55%, after which little further improvement took place that season. In regard to the relative resistance of the varieties to degrading by frost exposure, it is difficult to make any comparison because of the variations in freezing temperature, and the point will be left for consideration in connection with the 1932 series.

The weight per measured bushel of the grain, taken after curing and threshing, reached a maximum at the same stage at which the grades of the check samples became relatively constant. The weight is of course a factor in determining the grade. There was very little difference in the weights of frozen and non-frozen samples. In a few cases, where the grade was materially reduced by the frost exposure, the frozen wheat was slightly lighter. This agrees with common experience in regard to frosted wheat, the explanation usually offered being that the wrinkling or blistering of the kernels prevents such close packing in the bushel measure.

While it is not intended in this paper to discuss the seed value of frosted wheat, the laboratory germination percentages of the samples are given in

Table II because they show an interesting difference in the behavior of Garnet as compared with the other varieties. The frost exposures were not severe enough to injure significantly the germination of any of the varieties. In the case of Garnet alone, the germination of every sample was improved by the freezing treatment, a result for which no explanation can be offered at present, though it suggests a field for enzyme investigations.

TABLE II

Germination in relation to stage of maturity and exposure to frost. Series of 1929

Degrees of frost, F.		Dry matter at cutting, %	Germination, %			Dry matter at cutting, %	Germination, %		
A	*B*		*A*	*B*	Check		*A*	*B*	Check
		Garnet				Reward			
7	4	43.4	92	95	80	41.5	88	84	84
5	3	45.5	94	95	91	42.9	90	87	92
8	6	49.0	98	95	87	49.0	94	91	95
5	3	49.5	97	94	89	48.2	94	90	99
4	2	57.4	99	97	83	52.6	99	99	95
8	6	59.6	96	92	90	54.3	99	94	97
9.5	8	60.6	98	96	86	56.6	94	95	89
14	9	65.0	93	95	84	63.0	91	90	95
		Marquis				Renfrew			
4.5	3	46.8	98	98	98	40.5	100	98	99
8	6	48.8	98	98	99	46.2	100	99	96
9	7	53.7	98	97	98	50.1	98	100	93
9.5	8	54.9	100	99	99	52.7	99	94	88
9	7	56.7	97	96	99	54.4	93	92	74
8	6	57.6	98	98	98	52.3	87	95	97
13	11	58.8	94	94	97	57.7	93	95	92
9	7	59.0	93	92	99	56.5	84	89	86
7	5	66.6	98	98	98	60.5	91	90	83

Series of 1930

One feature of the 1930 samples requires explanation. The north end of the rather long, narrow block of Marquis wheat, five acres in extent, grown for this experiment, was exposed to soil blowing in the spring which cut back the young plants, then 2 or 3 in. tall, delaying their development and causing uneven ripening. The wheat in the more protected south end was in flower before that in the north end had finished heading. The first 10 cuttings were made from the south end, and the remainder were made some from the north end and some from the south end, but the wheat from the two ends was never mixed. In the tables, the samples are listed in the order of their dates of cutting, with those from the north end indicated by an asterisk. The grade, weight per measured bushel and weight per 1000 kernels of the 1930 samples, as affected by the freezing temperatures indicated at progressive stages of maturity, are shown in Table III.

TABLE III

GRADE, WEIGHT PER BUSHEL AND WEIGHT PER 1000 KERNELS OF MARQUIS WHEAT IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST (°F.). SERIES OF 1930

Dry matter at cutting, %	Grade**			Weight per measured bushel, lb.			Weight per 1000 kernels gm. (13.5% moisture)		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	*F*	*F*	*F*	43.0	45.0	43.0	13.50	13.59	12.86
34.9	*F*	*F*	*F*	46.5	49.5	49.5	16.58	18.18	16.99
39.4	*F*	*F*	*F*	50.5	52.0	53.0	19.75	20.55	19.55
43.8	*F*	5	4	55.5	57.0	57.5	23.38	24.28	23.77
46.8	*F*	5	4	57.5	57.5	58.0	26.13	25.64	25.89
48.3*	*F*	6	6	56.5	55.0	56.5	23.89	26.46	26.29
50.5*	*F*	*F*	5	55.5	57.0	59.5	26.47	28.91	28.43
51.1*	*F*	—	5	57.5	—	60.0	26.92	—	29.56
51.3*	*F*	6	5	58.5	58.5	58.5	29.21	28.05	27.12
55.8*	*F*	*F*	4	61.5	61.5	62.5	31.53	32.13	32.70
56.8	6	4	3	62.0	63.5	64.5	35.34	35.94	35.29
57.3	6	4	1	63.0	64.0	65.0	37.68	36.01	36.43
58.1*	5	—	3	61.0	—	62.0	35.10	—	35.50
59.1*	6	—	4	62.0	—	64.0	36.11	—	35.55
65.5*	3	—	3	63.5	—	63.5	35.97	—	33.18
65.6	5	—	2	62.5	—	64.5	36.96	—	36.96
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	*F*	*F*	*F*	43.0	43.0	48.0	14.54	14.32	15.50
38.1	*F*	*F*	6	47.5	49.0	51.0	16.85	16.91	18.50
40.9	*F*	*F*	4	54.0	53.5	56.0	21.20	21.18	22.20
45.5	*F*	*F*	4	57.0	57.0	58.5	23.64	24.23	25.40
46.7	*F*	*F*	4	59.0	58.5	60.0	26.59	27.24	26.98
50.5*	*F*	*F*	5	59.0	56.0	58.0	27.76	24.93	26.34
51.1*	*F*	—	5	59.5	—	57.5	26.88	—	29.56
53.3*	*F*	*F*	5	61.0	60.0	59.5	29.50	28.19	28.70
54.9	*F*	*F*	4	62.5	62.0	61.5	34.40	33.08	32.63
57.4*	*F*	*F*	3	61.0	61.0	62.5	33.08	33.90	32.39
58.1*	6	—	3	61.5	—	62.0	34.89	—	35.50
58.5	5	5	2	62.0	62.5	65.0	36.60	36.38	36.00
59.1*	6	—	4	62.0	—	64.0	35.73	—	35.55
65.5*	5	—	3	63.0	—	63.5	36.85	—	33.18
65.6	5	—	2	62.0	—	64.5	35.66	—	36.95
69.4	—	—	1	—	—	65.0	—	—	36.31

* *Samples from north end of the field.* ** *F = Feed.*

The earlier stage at which sampling was begun, as compared with 1929, and the more severe freezing treatment are reflected in the grades. The check samples improved as before with the progressive development of the kernels, though the official grades do not differentiate between them until a dry matter content of about 40% at cutting is reached. Below that they are all grouped together as "Feed". The upper limit of grade improvement is reached at a dry matter content of about 57%, as compared with about 54% for Marquis in 1929. The samples from the north end graded lower than those from the south end harvested at corresponding dry matter contents, because of the lack of uniformity and the presence of green kernels in the former, arising out of the uneven development of the crop in that part of the field.

Where the check sample is graded "Feed", it is of course impossible to judge frost effects by the grade of the corresponding frosted samples. In all other cases but one, an exposure to even four degrees of frost caused a lowering in grade. Such a relatively light frost for the most part caused no injury to the grade in the 1929 series, but the duration of exposure at the minimum temperature in any given run that year was only one to two hours as compared with four hours in 1930. It must not be concluded, however, that a night temperature falling to 28° F. and remaining there even a short time would not under natural conditions injure the grades of immature wheat standing in the field with each head exposed to maximum radiation on all sides. Experimental conditions cannot duplicate natural conditions exactly, but have the advantage of producing a series of comparable samples of which the history is fully known. An exposure to 8, 10 or 14 degrees of frost reduced to "Feed" all samples but two up to a dry matter content of 57.4%, the point at which the check samples attained their maximum grade value. The exceptions were two of the uniformly maturing samples from the south end of the field, with 56.8 and 57.3% dry matter, which after an exposure to eight degrees of frost graded No. 6. This is not to say that exposures to 8, 10, or 14 degrees of frost were equal in their effects, but merely that all the more immature samples so treated were thrown into the lowest grade, and no further differentiation can be made on this basis. In the more mature samples some difference in effect can be seen, but it is still relatively slight, owing in part to the narrower range of effect produced at this more resistant stage. But with one exception even the most mature samples were cut two or three grades by exposure to either 8 or 14 degrees of frost..

The weight per measured bushel recorded in Table III was determined after the samples had been stored together in a cool room for about four months, at which time they all contained close to 14% moisture. In earlier work in the laboratories co-operating with the Associate Committee on Grain Research, the practice had been tried of determining the weight per bushel when the wheat had been tempered to 13% moisture in preparation for milling. It was soon found that the weights so determined were so much influenced by the previous history of the samples, particularly by their moisture content just before tempering, as to be unreliable. The practice now is to determine both weight and moisture content of samples as received in the laboratory, and if possible to handle all the samples in a given series in such a way that they may be kept at about the same moisture content and never dried excessively before weighing.

The weight per bushel of the check samples rose from 43 to 65 lb. during the experiment, reaching the latter maximum when the kernels contained 57 to 58% dry matter at the time of cutting. Again, as in 1929, the attainment of maximum weight coincided roughly with the attainment of maximum grade. Unlike the 1929 freezing treatment, which had little effect on weight, the more rigorous exposures of 1930 lowered the weight appreciably in most cases. The exceptions may perhaps be attributed to sampling errors.

The weight per 1000 kernels, corrected in Table III to a moisture content of 13.5%, followed very much the same course in relation to stage of development as did the weight per bushel, reaching a maximum at the same dry matter content, namely, 57 to 58%. There are no differences in either check or frosted samples after this point which may not reasonably be laid to sampling errors. Differences between the check and frozen immature samples are considered to involve a further factor, namely, the balance between translocation and respiration after the treated grain had been stooked for curing. Exposure to 10 and 14 degrees of frost is assumed to stop both these processes very largely, and the first four samples so treated are definitely lighter than their checks, apparently having lost more by the stoppage of translocation than they saved by the stoppage of respiration. The samples exposed to 4 and 8 degrees of frost are irregular in weight, but on the whole just as heavy as their checks, indicating that these exposures checked translocation and respiration only partially, if at all. More data on translocation and respiration in stooked grain are given in the next paper of this series.

Physical Classification of Kernels

A physical classification of the kernels in the 1930 samples, similar to that described in the report by Geddes, Malloch and Larmour (2) reviewed briefly in the introduction to this paper, was carried out by an experienced grader formerly on the staff of the Western Grain Inspection Division. Approximately 1000 kernels of each sample were classified, kernel by kernel, as vitreous, starchy, immature, green, bran frosted, or heavily frosted. All kernels showing blisters or "scalding" were classified as frozen. Those on which the blistering was carried down into the crease were grouped under *heavy frost*, all others under *bran frost*. *Green* kernels were very immature and shrunken, with the green color persisting. *Immature* kernels did not show external evidence of frost, but could not be classified as green or sound; they are commonly bronze or pinkish in color. Sound kernels were divided into *vitreous* and *starchy*, the latter class including any noticeably piebald kernels.

The results of this classification are given in Table IV. The proportions of the various classes, though first determined by number, are reported in percentages by weight, since this is the basis used in the official grade specifications.

This method of classification proved to be inapplicable to very immature wheat, since it is impossible to distinguish sharply between kernels which are shrunken merely from lack of development and those which are also frost injured. The percentage of so-called green kernels is in fact increased by more severe freezing, at the expense partly of the frozen class, because of the "fixing" of the chlorophyll brought about by the freezing treatment. This effect is very noticeable in the second sample, cut at 34% dry matter. The same difficulty occurs, though in less degree, with the more mature samples, in a number of which the apparent percentage of heavily frosted

TABLE IV

PHYSICAL CLASSIFICATION OF MARQUIS WHEAT KERNELS, BY WEIGHT, IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1930

Dry matter at cutting, %	Degrees of frost, F.	Vitreous, %	Starchy, %	Total sound, %	Immature, %	Green, %	Bran frost, %	Heavy frost, %	Total frost, %	Grade**
31.2	8	0	0	0	82.1	2.9	0	14.9	—	F
	4	0	0	0	96.2	0.5	0	3.3	—	F
	None	0	0	0	98.9	0.5	0	0.5	—	F
34.0	13.5	0	0	0	53.4	27.0	0	19.6	—	F
	9	0	0	0	67.1	16.7	0	16.1	—	F
	None	0.6	0	0.6	97.0	0.9	0	1.5	—	F
34.9	8	0.8	0	0.8	81.9	4.2	0	13.0	—	F
	4	2.0	0	2.0	93.0	0.9	0	4.1	—	F
	None	1.8	0	1.8	97.6	0.6	0	0	—	F
38.1	14	0	0	0	61.8	4.6	1.3	32.3	—	F
	10	1.1	0	1.1	74.5	4.4	0.8	19.3	—	F
	None	6.0	0	6.0	93.3	0.7	0	0	—	6
39.4	8	0	0	0	62.3	4.4	0	33.2	—	F
	4	0	0	0	78.4	0.6	5.5	16.4	—	F
	None	1.8	0	1.8	97.8	0.3	0	0	—	F
40.9	14	0	0	0	55.9	9.3	2.6	32.2	—	F
	10	0.5	0	0.5	51.6	7.5	3.7	36.7	—	F
	None	8.2	1.2	9.4	90.0	0.6	0	0	—	4
43.8	8	2.4	0	2.4	50.1	6.2	4.9	36.3	—	F
	4	5.2	1.5	6.7	60.9	1.8	3.8	26.8	—	5
	None	10.3	2.2	12.5	85.8	1.8	0	0	—	4
45.5	14	0	0	0	47.3	17.3	7.5	27.9	48.8	F
	10	3.1	0	3.1	56.1	10.2	5.6	25.0	45.7	F
	None	48.8	0	48.8	50.9	0.3	0	0	—	4
46.8	8	5.3	0	5.3	44.9	10.0	0	39.8	43.4	F
	4	12.9	0	12.9	46.2	1.8	14.2	24.9	35.8	5
	None	48.7	0	48.7	49.2	2.1	0	0	—	4
46.7	14	1.1	0	1.1	58.4	8.0	4.5	27.9	43.1	F
	10	2.2	0	2.2	50.7	6.9	3.3	36.9	42.0	F
	None	42.6	1.6	44.2	54.0	1.8	0	0	—	4
48.3*	8	0	0	0	39.8	10.1	2.5	47.7	—	F
	4	2.0	6.0	8.0	56.1	1.4	8.2	13.8	—	6
	None	6.8	4.8	11.6	76.4	11.3	0.6	0	—	6
50.5*	14	0	0	0	47.1	14.4	5.1	33.4	78.7	F
	10	0	0	0	38.4	5.8	1.0	54.9	78.8	F
	None	72.1	6.6	78.7	17.9	3.4	0	0	—	5
50.5*	8	0	0	0	35.2	11.1	3.8	50.0	80.6	F
	4	0	0	0	19.3	5.8	10.4	64.4	80.5	F
	None	79.1	1.3	80.4	13.6	5.9	0	0	—	5
51.1*	14	0	0	0	36.0	11.6	3.8	48.6	80.5	F
	7	0	0	0	27.3	10.7	0.8	61.2	80.5	F
	None	79.7	0.8	80.5	15.4	4.1	0	0	—	5
51.3*	8	0	2.2	2.2	24.4	7.7	0	65.7	78.4	F
	4	14.6	0	14.6	27.3	10.9	22.6	24.5	65.9	6
	None	78.9	1.8	80.7	12.4	7.0	0	0	—	5

TABLE IV—*Concluded*

PHYSICAL CLASSIFICATION OF MARQUIS WHEAT KERNELS, BY WEIGHT, IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1930

Dry matter at cutting, %	Degrees of frost, F.	Vitreous, %	Starchy, %	Total sound, %	Immature, %	Green, %	Bran frost, %	Heavy frost, %	Total frost, %	Grade**
53.3*	14	0	0	0	44.4	13.4	2.1	40.0	85.1	*F*
	10	0.6	0	0.6	40.6	12.7	0.8	45.3	84.6	*F*
	None	83.1	2.1	85.4	11.9	2.9	0	0	—	5
54.9	14	2.0	0	2.0	12.6	2.2	12.4	71.0	91.3	*F*
	10	0	0	0	5.1	3.4	5.7	85.7	93.0	*F*
	None	90.5	2.1	92.6	4.4	2.5	0	0.4	—	4
55.8*	8	1.4	0	1.4	16.4	7.0	6.9	68.3	91.8	*F*
	3	5.2	0	5.2	8.4	4.4	2.6	79.4	88.0	*F*
	None	91.4	1.5	92.9	5.9	0.9	0	0	—	4
56.8	8	7.4	0.3	7.7	11.3	7.7	10.2	63.0	90.6	6
	4	64.4	1.0	65.4	6.7	0.6	6.1	21.1	32.9	4
	None	96.4	1.5	97.9	1.5	0.1	0	0.4	—	3
57.3	8	6.6	1.4	8.0	1.4	0.1	5.5	85.0	91.0	6
	4	36.6	5.3	41.9	0.9	0.6	14.8	42.3	57.6	4
	None	92.2	6.8	99.0	1.0	0	0	0	—	1
57.4*	14	3.3	0	3.3	9.6	12.7	16.9	57.5	86.6	*F*
	10	3.0	0.6	3.6	17.4	9.6	15.5	53.9	86.3	*F*
	None	87.5	2.4	89.9	9.9	0.2	0	0	—	3
58.1*	14	12.6	0	12.6	14.8	5.1	7.5	59.9	78.3	6
	7.5	14.9	8.3	23.2	11.9	2.1	19.0	43.8	69.3	5
	None	66.1	8.5	74.6	8.6	0.4	16.4	0	—	3
58.5	14	9.9	1.9	11.8	1.5	0.4	4.3	82.0	86.3	5
	10	11.2	0	11.2	0.6	0.6	3.9	83.7	86.9	5
	None	95.3	2.3	97.6	1.9	0	0	0.5	—	2
59.1*	14	9.4	0	9.4	3.5	7.4	10.5	69.1	81.2	6
	8	16.9	0	16.9	2.3	4.3	12.1	64.4	73.8	6
	None	86.2	2.6	88.8	9.3	0	1.9	0	—	4
65.5*	14	24.6	0	24.6	2.2	5.7	16.1	51.4	71.5	5
	7	76.3	5.1	81.4	4.7	0.7	11.8	2.1	15.8	3
	None	92.0	3.1	95.1	3.9	0	1.0	0	—	3
65.6	14	10.9	0	10.9	1.1	2.9	9.4	75.7	86.7	5
	7	19.9	0.4	20.3	2.2	1.8	7.2	68.5	77.3	5
	None	95.3	2.3	97.6	2.4	0	0	0	—	2
69.4	None	96.7	2.6	99.3	0.6	0	0	0	—	1

* *Samples from north end of field.* ** *F = Feed.*

kernels bears an *inverse* relation to the severity of freezing exposure. Here an attempt was made to show the real effect of frost by inserting in Table IV the column headed "Total frost". This is calculated by adding together the percentages of immature, green, bran frost and heavy frost in the samples exposed to frost, and subtracting the sum of the immature and green in the

unfrozen check samples. The device was only partially successful since, with a few exceptions, the values for total frost were too constant to provide a good basis of differentiation.

Up to a dry matter content of 40% at cutting, nearly all the kernels of the unfrozen checks were included in the immature class. The traces of frost indicated in the first two and in a few later check samples are probably attributable either to imperfect cleaning of the thresher between samples or to error in judgment in classifying.

At least a few sound kernels were found in all check samples but the first one, though the percentages were negligible till the grain reached a dry matter content of 40% at cutting. After this they rose rapidly, nearly half the kernels being sound at 45 to 47% dry matter, although that corresponded to the milk stage. This shows the considerable development in the stook while drying. From the time the grain contained 50% dry matter at cutting, at least 80% of the kernels were sound in most samples. The percentage was somewhat influenced by the section of the field from which the samples were cut, those from the north end containing a higher percentage of green and immature kernels, owing to the delayed development and uneven ripening of the crop already described. In all samples, the sound kernels were mainly vitreous, the highest proportion of starchy kernels being 8.5%.

The lack of uniformity in the north end samples was such an important factor in grading as largely to obscure the relation between percentage of sound kernels and grade in the unfrozen checks. In the frozen samples, notwithstanding the more limited operation of the same factor, the relation between percentage of sound kernels and grade is fairly evident in the following brief census:

Feed grade—	18 samples	contained	no sound kernels	
	16 "	"	0.5 to 5.3%	sound
Grade No. 6—	7 "	"	7.7 to 16.9%	"
" " 5—	8 "	"	6.7 to 24.6%	"
" " 4—	2 "	"	41.9 and 65.4%	"
" " 3—	1 "	"	81.4%	"

(No frozen samples graded higher than No. 3 Northern.)

As already indicated, the study of Geddes, Malloch and Larmour (2) on commercial samples led to the conclusion that, of the various quantities derived from the physical classification of kernels, only the percentage of sound kernels (or of its best fraction, the vitreous kernels) promised usefulness as a grading factor. They were working with heterogeneous samples, and it seemed possible that with the basically homogeneous material of the 1930 artificially frozen series more precise results might be obtained. It is doubtful, however, that any of the classes shown in Table IV are worthy of consideration as an index of quality, other than the sound kernels.

The limitations of any grading system based wholly on the physical characteristics of the kernels appear plainly in the degradation of the most mature samples exposed to frost. The sample cut when it contained 65.6% dry

matter, a stage riper than necessary for normal harvesting with a binder, had over 75% of its kernels marked by exposure to seven degrees of frost, and was cut three grades, without any corresponding injury to quality which could be detected by chemical or baking tests.

Series of 1932

The effect of freezing at different stages of maturity on the grade and test weight of Garnet, Reward, Red Bobs and Marquis samples produced in 1932 is shown in Table V and Fig. 1. These varieties during the seven-year period

TABLE V

EFFECT OF FREEZING AT DIFFERENT STAGES OF MATURITY (AS SHOWN BY DRY MATTER CONTENT OF KERNELS AT TIME OF CUTTING) ON THE GRADE AND TEST WEIGHT. SERIES OF 1932

Date of cutting	Degrees of frost, F.	Garnet			Reward			Red Bobs			Marquis		
		Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.
Aug. 4	None	51.1	3	63.5	46.2	4	64	—	—	—	—	—	—
	10		*F*	62		*F*	62	—	—	—	—	—	—
Aug. 6	None	56.2	3	64	50.7	3	65.5	—	—	—	—	—	—
	4		3	64		3 *p*	65	—	—	—	—	—	—
	10		5	64		*F*	64.5	—	—	—	—	—	—
Aug. 9	None	70.8	2	63.5	57.6	2	66.5	—	—	—	—	—	—
	4		2	63.5		2 *g*	66	—	—	—	—	—	—
	10		3 *g*	64		5 *p*	65	—	—	—	—	—	—
Aug. 13	None	70.9	2	64	58.2	1 *H*	66.5	55.0	1 *H*	65	57.2	2	63.5
	4		2	64		3	66		1	64.5		4 *g*	63.5
	10		3	64		5 *p*	65		6	61		*F*	63
Aug. 15	None	78.5	1	64	62.7	1 *g*	66.5	55.4	1 *H*	65	60.5	1	64.5
	4		1	64.5		3	66		2	65		2	64
	10		2 *p*	64.5		4	65.5		6	62		*F*	63
Aug. 17	None	80.5	1	64	75.1	1 *H*	66	59.6	1 *g*	65	62.8	1	65
	4		1	64		1 *H*	66		2	65		2	65
	10		2	64		2	66		5 *g*	64		*F*	63
Aug. 19	None	84.8	1	64.5	78.5	1 *H*	66	62.4	1 *H*	65	76.5	1 *H*	65
	4		1	64.5		1 *H*	66		1 *H*	65		1 *H*	65
	10		(1)	64		1 *H*	66		3 *p*	65		6	64
Aug. 22	None	85.7	1	64.5	86.8	1 *H*	66	68.8	1 *H*	65	78.4	1 *H*	65.5
	4		1	64		1 *H*	66		1 *H*	65		1 *H*	65.5
	10		(1)	64		1 *H*	66		1	65		5	64
Aug. 24	None	—	—	—	—	—	—	74.4	1 *H*	65	81.9	1 *H*	65.5
	4	—	—	—	—	—	—		1 *H*	65		1 *H*	65
	10	—	—	—	—	—	—		1	65		2 *g*	65
Aug. 26	None	—	—	—	—	—	—	82.2	1 *H*	65	89.3	1 *H*	65
	4	—	—	—	—	—	—		1 *H*	65		1 *H*	65
	10	—	—	—	—	—	—		1 *H*	65		1 *p*	65

* *1 H = 1 Hard; F = Feed; g = good; p = poor.*

1926-32 at Edmonton had an average growing season of 101, 102, 104, and 107 days respectively. In the particular year of this experiment (1932) the periods were 94, 96, 98 and 99 days. Their relative earliness is reflected in the dry matter content of the kernels at any date of cutting shown in Table V, except that the positions of Red Bobs and Marquis are unexplainably reversed. The sensitivity of the two latter to frost damage on any given date, as indicated by the effect on the grades shown in the table, is however proportional to their recognized habits of maturation rather than to the moisture content of the kernels. This, it may be remarked in passing, is the only instance encountered throughout these investigations where dry matter content did not appear to be a reliable index of maturity.

An arbitrary factor interfered with the interpretation of the grades as first recorded, in that the grading regulations exclude Garnet from grades higher than No. 2 Northern because of varietal differences in quality. The Garnet samples were regraded with this limitation removed, but unfortunately the last two samples were missed on this occasion, and the grade No. 1 Northern indicated in brackets is a presumption based on the trend of preceding samples in comparison with the corresponding trend in other varieties.

Exposure to four degrees of frost, up to about 60% dry matter, cut some samples of Reward and Red Bobs and all samples of Marquis one or two grades. Only one sample of Garnet so exposed came within this dry matter category, and it was unaffected. Exposure to 10 degrees of frost cut the grades of Reward and Red Bobs up to a dry matter content of about 75%, of Garnet up to about 80%, and of Marquis over its entire dry matter range, which extended to 89.3%. This heavier frost differentiated Marquis from the other varieties, the former being apparently more sensitive to degradation by frost during the whole maturation period (Fig. 1). The differences between the other three varieties are probably not significant.

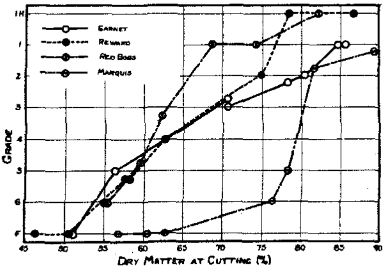

FIG. 1. *Grades of four wheat varieties exposed at progressive stages of maturity to 10 degrees of frost for four hours.*

The reduction in grade by exposure to four degrees of frost was accompanied either by no change or only a very slight reduction in weight per bushel. The more severe blistering caused by the 10-degree frost had in several, though not in all, cases an appreciable effect on weight per bushel. The earlier samples, as might be expected, were more reduced in weight than the later ones.

In the unfrozen checks the weight per bushel reached a maximum in samples containing 55 to 60% dry matter at cutting, a stage perhaps slightly later than in the corresponding varieties in 1929 and 1930. The grades of Reward and Red Bobs also reached their best in this dry matter range, but Garnet and Marquis improved one more grade at a later stage. This may be accounted for in Garnet by the removal in 1932 of the arbitrary factor in its grading already referred to, while in Marquis the change from No. 1 Northern to No. 1 Hard (a grade attained only in 1932) has but restricted significance, since in these high grades discrimination is quite rigid and differences are comparatively small.

There is on the whole a good agreement in the results of all three seasons with respect to the stage at which grade and weight per bushel of unfrozen wheat reached an approximately constant level. Susceptibility to degradation by exposure to frost continued in all cases for a considerable time thereafter.

The extent to which such superficial injury, unaccompanied by any changes in internal quality which can be detected by chemical or baking tests, affects the real value of the wheat, is a very difficult point to assess. When blistering is severe enough to reduce the weight per bushel, it is probably safe to assume that the milling yield of flour will also be reduced. There seems little doubt, however, that the heavy cut in grade resulting from superficial frost injury is often disproportionate to the real change in commercial value.

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AMIDE NITROGEN IN GERMINATING SEEDS[1]

By A. G. McCalla[2]

Abstract

Experiments with cereal seeds indicate that the amide groups of reserve seed proteins are more readily removed from the protein during germination than are the other structural units of the protein. No accumulation of amides was found in the non-protein nitrogen of seeds nor in seedlings.

Introduction

There is a considerable volume of literature dealing with the distribution of nitrogen fractions in germinating seeds, but little of it refers to the cereals. With regard to the amide fraction of the nitrogen, Kostychev (3) sums up the older work as follows: ". . . . it can be considered as established that only a small quantity of amides are formed directly by the hydrolysis of reserve proteins. Most of them are the result of synthesis at the expense of the other crystalline structural units of the protein molecule." Kostychev's review covered work by Schulze, Prianischnikov, Zaleski, Butkewitsch and others, all of whom studied principally legume seeds. In one experiment it was reported that 73% of the nitrogen from decomposed proteins was accounted for by the amide asparagine.

Kostychev's conclusion has not been entirely confirmed by more recent work, even when its application is limited to legumes. It is important to note that most of the results he considered were obtained in studies in which the materials analyzed had been previously dried at various temperatures. He cites the work of Schulze and his school as outstanding in the field, but Schulze and Castoro (6) dried most of their material at 60 to 70° C., under which conditions Chibnall (2) has shown that the asparagine content is greatly increased as a result of the activity of enzymes.

Sure and Tottingham (7) found that amides accumulated in the shoots of germinating peas, but in much smaller amounts than had been reported previously. More recently, Bonnet (1) studied lupin seeds and seedlings during germination and found that the decrease in protein nitrogen of seeds could be accounted for by increases in amide and amino nitrogen. The total increase in amino nitrogen, however, was greater than that in amide nitrogen.

None of these investigators determined the composition of the proteins remaining undecomposed at the time the other analyses were made. Chibnall

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(2) found that enzymatic decomposition of protein during low-temperature drying of leaves decreased the amount of protein but did not change the character of that portion remaining undecomposed.

Sasaki (5) fractionated the heat-coagulable protein of germinating soy bean seeds. Although he presents no data, he states that no intramolecular change of the protein was indicated from his results.

Detailed experiments with cereal seeds are scarce. Takahashi and Shirahama (8) germinated hulless barley and studied the nitrogen distribution. Unfortunately they also dried their material before analysis. They found a decrease in protein during germination, and since this decrease was particularly great with the alcohol-soluble protein, they prepared hordein from germinated and ungerminated seeds. They then fractionated the preparations, and their results indicated no significant change in composition during germination. It must be noted, however, that none was to be expected, as any marked change in composition would probably change the physical properties to such an extent that the altered portion would be lost during preparation.

A general survey of the literature leaves some doubt as to the validity of generalizing Kostychev's conclusion to cover all seeds. During the course of work on nitrogen metabolism of cereals, the writer obtained evidence that the amide groups of reserve proteins were more readily removed from the protein during decomposition than were the other nitrogen groups. It was thought that this relation might hold for decomposing seed proteins during the early stages of germination. If it did, it would offer an alternative explanation for the accumulation of non-protein amides noted by other workers in this field. It is believed that the results presented in this paper indicate a need for revising Kostychev's conclusion, at least in its application to cereals.

Methods

Wheat, barley, rye, corn and pea seeds were studied. The seeds were soaked overnight in tap water, then spread out on cheesecloth over water. Two samples of wheat (1*b* and 2*b*) were allowed to germinate until the seedlings just began to split the seed coat, and then were analyzed. All other samples were allowed to germinate three days, after which the seedlings were separated from the seeds, and the latter extracted and analyzed. The methods used for extraction and analysis have been fully described in another paper (4). In this experiment protein nitrogen, and amide, humin, and basic fractions of the protein were determined for the majority of samples. The non-protein nitrogen of two pairs of wheat samples (1*a* and *c*, 2*a* and *c*) were fractionated, and two lots of wheat seedlings were analyzed.

In separating the seedling from the seed it was impossible to get an exact separation, because parts of the seedlings were contained within the seed coat. In every case the procedure adopted was to cut the seedling from the seed, leaving with the seed all those parts inside the seed coat. Thus the total

protein decreases in germinated seed recorded in Table I are too low, because some of the protein nitrogen recorded as undecomposed protein had been regenerated in the part of the seedling left within the seed.

Results

The results of protein analyses are presented in Table I. The decrease in protein nitrogen during germination has been noted by all other workers in this field. The humin results are not presented because any differences occurring are less than the experimental error. Basic nitrogen expressed as percentages of the protein nitrogen undergoes a slight increase during germination. When expressed in terms of weight changes, although there were apparently some decreases and some increases, these were negligible as compared with the changes in amide nitrogen.

In marked contrast to the results for humin and basic nitrogen, those for the amide fraction of the protein show that the decrease in this fraction accounts for most of the decrease in protein nitrogen. Some of the results, in fact, indicate that there was more amide nitrogen split from the protein than there was protein decomposed. These results, obtained only with wheat, are probably explained by the incomplete separation of seedlings from the seeds as described under Methods. It is possible that some intramolecular change, by which some of the amide was converted to a non-amide form, took place within the reserve protein itself, but this does not seem likely. Despite the inaccuracies of the methods, however, there can be no doubt that the total amide nitrogen of the protein was greatly reduced during germination.

The results for non-protein nitrogen of seeds and for total nitrogen of seedlings are presented in Table II. They do not indicate any accumulation of amides at the expense of the other structural units of the reserve protein. In this experiment, the other nitrogen compounds must have increased at the expense of the amide split from the protein reserves. The total amide nitrogen present in the non-protein nitrogen of seeds and in seedlings accounts for only 15%, in the case of sample *1c*, and 25%, in the case of sample *2c*, of the amide removed from the seed proteins.

The results with peas indicate no removal of amide groups during germination. The metabolism of legumes is known to be quite different from that of cereals, and the conclusions drawn by workers who have confined their attention to these plants, cannot be refuted by the results of this experiment.

The results suggest, however, that it is necessary to modify the general conclusions which have been applied to all types of seed plants. The results obtained with wheat are definite, and those with all other cereals support the conclusion that the amide groups of the reserve proteins are more readily removed during the early stages of germination than are the other structural units.

TABLE I

NITROGEN DISTRIBUTION IN UNGERMINATED AND GERMINATED SEEDS

Sample No.	Seed	Condition	Total nitrogen, %	Protein nitrogen			Amide nitrogen of protein				Basic nitrogen of protein			
				% of total N	Mgm. per 1000 seeds	Decrease in germinating, mgm.	% of protein N	Mgm. per 1000 seeds	Decrease in germinating: Mgm.	Decrease in germinating: % of protein N decrease	% of protein N	Mgm. per 1000 seeds	Decrease in germinating: Mgm.	Decrease in germinating: % of protein N decrease
1a	Wheat	Ungerminated	3.74	93.3	1017	—	18.1	184	—	—	32.1	326	—	—
b	Wheat	Germinated	3.74	90.6	987	30	14.3	144	40	133	—	—	—	—
c	Wheat	Germinated	3.74	85.6	932	85	6.5	61	123	145	34.2	319	7	8
2a	Wheat	Ungerminated	2.90	93.8	949	—	13.2	125	—	—	32.2	306	—	—
b	Wheat	Germinated	2.90	90.2	913	36	11.5	105	20	56	—	—	—	—
c	Wheat	Germinated	2.90	87.3	883	66	6.6	58	67	102	31.8	281	25	38
3a	Wheat	Ungerminated	2.58	94.1	888	—	13.4	119	—	—	31.6	281	—	—
b	Wheat	Germinated	2.58	88.9	858	30	6.6	57	62	207	32.9	282	−1	−3
4a	Hulled barley	Ungerminated	2.21	95.6	982	—	14.8	145	—	—	—	—	—	—
b	Hulled barley	Germinated	2.21	89.7	921	61	12.6	116	29	48	—	—	—	—
5a	Hulless barley	Ungerminated	2.99	93.3	760	—	15.6	119	—	—	18.5	141	—	—
b	Hulless barley	Germinated	2.99	88.4	720	40	12.6	91	28	70	20.4	147	−6	−15
6a	Rye	Ungerminated	2.83	87.5	456	—	14.4	66	—	—	19.8	90	—	—
b	Rye	Germinated	2.83	81.4	424	32	13.1	55	.11	34	21.3	90	0	0
7a	Corn	Ungerminated	1.60	91.0	291	—	12.8	37	—	—	—	—	—	—
b	Corn	Germinated	1.60	89.2	285	6	11.8	33	4	67	—	—	—	—
8a	Peas	Ungerminated	3.33	90.4	458	—	7.2	33	—	—	—	—	—	—
b	Peas	Germinated	3.33	86.7	440	18	7.9	35	−2	−11	—	—	—	—

TABLE II

AMIDE NITROGEN IN NON-PROTEIN NITROGEN OF WHEAT SEEDS AND IN TOTAL NITROGEN OF SEEDLINGS

Sample No.	Condition of seed	Non-protein nitrogen of seeds		Amide N of seed non-protein nitrogen			Total nitrogen of seedlings		Amide N of seedling total nitrogen		Mgm. of amide N produced from protein of seeds in germinating
		% of T.N. of dry seeds	Mgm. per 1000 seeds	% of non-protein N	Mgm. per 1000 seeds	Mgm. increase in germinating	% of T.N. of dry seeds	Mgm. per 1000 seeds	% of T.N. of seedlings	Mgm. per 1000 seeds	
1a	Ungerminated	6.7	73	17.0	12	—	—	—	—	—	—
c	Germinated	14.4	157	13.4	21	9	8.2	90	10.2	9	18
2a	Ungerminated	6.2	63	13.5	9	—	—	—	—	—	—
c	Germinated	12.7	128	12.7	16	7	10.3	104	9.9	10	17

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ALBERTA BITUMEN

I. THE COMPOSITION OF BLOWN ALBERTA BITUMEN[1]

By Morris Katz[2]

Abstract

The relation between the physical consistency and chemical composition of a series of blown asphalts from Alberta bitumen has been studied. The amounts of asphaltous acids and anhydrides decrease on blowing, with rise in fusing point of the bitumen. The amounts of oily constituents and resins also decrease but the asphaltene content increases. The molecular weights of the resins increase from 733 to 1012 during blowing. The asphaltenes also show a pronounced increase in molecular weight from 2219 to 4690. Only traces of oxygen are found in the products blown for 6 to 8.5 hr. at 270° C.; the amount however increases in the later stages of blowing to a maximum of 1.88%. The oily constituents do not contain oxygen, which is distributed mainly in the resin fractions, with smaller amounts occurring in the asphaltene fractions. The sulphur and nitrogen in the bitumen are distributed principally among the resins and asphaltenes. The essential process in blowing bitumen with air is one of condensation by removal of some hydrogen to form water, followed by polymerization, assisted by the sulphur in the bitumen, to products of higher molecular weight. Products similar in composition and physical properties to commercial grades of mineral rubber have been prepared from Alberta bitumen by blowing.

Introduction

The present work was done as part of an investigation of the blowing of bitumen separated from the bituminous sand of Alberta, the immediate objective of the work being to secure from this semi-fluid natural bitumen products suitable for use as "mineral rubber."

It has been known for many years that marked changes may be induced in the physical properties of soft asphalts or residual oils derived from asphaltic, semi-asphaltic or non-asphaltic petroleums, by blowing with air at elevated temperatures. In 1876 W. P. Jenney (6) obtained a patent for treating sludge oil, resulting from petroleum refining, with a current of air at 250° C. Schall (19) proposed the use of oxygen for blowing paraffin, vaseline and heavy mineral oils at 140–150° C., in the presence of insoluble manganese compounds. Salathé (18) patented a process in which heated air was used for blowing asphalts either alone or in the presence of manganese dioxide or litharge. In 1894 Byerley (2) obtained asphalts by drawing air through petroleum residues at temperatures between 400° and 600° F. Soft grades, fusing under 200° F., were prepared by blowing for three days at 400° F., whereas the harder grades were produced by blowing for four to five days at 500° F. This method was used to manufacture blown asphalts on a commercial scale. Similar processes were developed for mixtures of petroleum residues and refined Trinidad asphalt or Gilsonite. Mixtures of air, carbon dioxide and steam were advocated for blowing the asphalt. However, Schreiber (20) showed that treatment with steam or carbon dioxide at

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280° C. left mineral oils practically unchanged, whereas air treatment caused a marked increase in the percentage of material insoluble in benzene. Although numerous patents were taken out involving the use of catalysts in blowing asphalts, Marcusson (14) expressed the opinion that the blowing process is not accelerated by the presence of a catalyst. The various methods in use for the preparation of blown asphalts have been reviewed by Abraham (1).

The chemistry of asphalts is extremely complex and but few data throwing light on their constituents are to be found in the literature. According to Marcusson (12, 13) asphalts may be broadly considered as consisting of a mixture of four groups of compounds; asphaltic acids and anhydrides, asphaltenes, resins and oily constituents. The methods of separating these components from asphalt, as developed by Marcusson, depend upon the fact that the acids and anhydrides are readily removed from a benzene solution of the asphalt by saponification with alcoholic potash solution and washing with water. The asphaltenes may then be precipitated from the benzene layer by adding petrolic ether. The filtrate contains the oils and resins, which are separated by treating the mixture with fuller's earth which adsorbs the resins and thus renders them insoluble in petrolic ether. The resins are then extracted with chloroform or benzene, and the oils with petrolic ether.

Some data are available on the composition of the oil fraction from asphalts. In 1898 Richardson (15) obtained a brown oil by distillation of Trinidad asphalt which on fractionation at a pressure of 60 mm. in an atmosphere of carbon dioxide yielded fractions indicating the presence of members of the $C_n H_{2n}$, $C_n H_{2n-2}$, and $C_n H_{2n-4}$ series. Mabery (9) in 1917 fractionated an oil obtained by the distillation of Gilsonite and Grahamite in an iron pipe at 400° C. and also showed the presence of hydrocarbons of the $C_n H_{2n}$ and $C_n H_{2n-2}$ series. In 1921 Krieble and Seyer (8) reported the results of a careful investigation of the oil fraction from a sample of Alberta tar sand. The sand was extracted with petrolic ether and the mixture of oil and resins thus obtained was treated with fuller's earth. The oil fraction was recovered by distilling off the petrolic ether. About 7.5 litres of this oil was obtained by extraction and subjected to fractional distillation under reduced pressure. A number of hydrocarbons were obtained corresponding to the series $C_n H_{2n}$, $C_n H_{2n-2}$, and $C_n H_{2n-4}$. The compounds were saturated and from a consideration of the molecular refraction it was deduced that they probably possessed ring structures of the mono-, di- and tri-cyclic naphthene type.

The resins and asphaltenes are derived from the oily constituents of the asphalt by condensation and polymerization and also by oxidation. The resins represent the intermediate product in the transformation of the oily constituents to asphaltenes. Their molecular weights are always higher than those of the oils from which they have been derived. Those isolated from blown Alberta bitumen contain oxygen, although the resins from the unblown material are free from oxygen. Marcusson and Picard (14) found that the resin content of a petroleum residue decreased on blowing with air

at 120–130° C. from 14.5% to 3.0%, whereas the asphaltenes increased from 17.5 to 34%. Further blowing at 170° C. increased the asphaltene content to 42.5%, the percentage of resins remaining substantially unchanged at 2.5%. The oily constituents decreased from 68 to 55%.

Sakhanov (Sachenen) (16, 17) investigated the properties of resins and asphaltenes derived from petroleum and its products. The resins contain oxygen and sulphur and are completely soluble in gasoline. Their mean molecular weight and specific gravity (between 1.01 and 1.04) are higher than those of the corresponding oil fractions from which they have been derived. These resins may be liquid, semi-liquid or solid, depending upon the molecular weight. According to Sakhanov, they have a highly developed polycyclic structure of the general formula $C_n\ H_{2n-m}\ O_p$ in which n may be 19–55, m 9–33, and p 2–3. They form true solutions, imparting an intense coloration to the solvent. The asphaltenes possess much higher molecular weights than the resins, although structurally these substances appear to be closely related. Sakhanov determined the molecular weight of asphaltenes isolated from various types of petroleum and obtained values of 5000 to 6000 by the cryoscopic method in benzene.

Recently, Mack (11) investigated the viscosity of asphaltenes derived from a blown Mexican asphalt with a softening point of 64° C. Solutions of asphaltenes in petrolenes, *i.e.*, mixtures of oil and resins, yielded values for relative viscosity which increased with falling temperature and also with increasing concentration. By using the relation log. relative viscosity = ckM, where c is the concentration in weight per cent, k, a constant, and M, the molecular weight, Mack obtained an average molecular weight of 1800 for the asphaltenes, from the relative viscosities at 120° C. At 25° C. values of 3480 to 4000 were obtained, and at 0° values as high as 5160 with asphaltenes from the same sample of blown asphalt. Mack ascribes this behavior to the fact that the asphaltenes are in true solution at 120° C. and that association of the molecules, due to their polar nature, takes place at lower temperatures. From the viscosity of sols in benzene, molecular weights of 1794 and 2312 were obtained at 25° and 7° C. respectively.

Chemical Composition of Blown Alberta Bitumen

In the present work, the writer has attempted to show the relation beween the physical consistency of a series of blown asphalts from Alberta bitumen, and their chemical composition. Starting with a product fusing at 34° C. (*R* and *B* method) as the raw material a series of blown products was prepared ranging in softening point from 60 to 169° C. The various bitumen samples were separated into the four main components, namely: (i) the asphaltous acids and anhydrides, (ii) asphaltenes, (iii) resins, and (iv) oily constituents. The amounts of acids and anhydrides decreased with rise in softening point of the bitumen from 1.85 to 0.42%. This is to be expected since at the blowing temperature used, 270–275° C., the saponifiable matter would be converted to unsaponifiable substances resembling asphaltenes. According to Abraham (1) this takes place above 200° C. The asphaltenes

increased from 24.2 to 57.8% in the bitumen of highest fusing point. This change appeared to take place more rapidly in the early stages of blowing, since bitumen fusing at 104° C. was found to contain 47.8% of asphaltenes. The amounts of oily constituents decreased to the same extent, from 55.0% in the raw bitumen to 37.8% in that fusing at 104° C., and finally to 29.1% in the material fusing at 169° C. The amounts of the resins, on the other hand, appeared to remain constant after decreasing from 15% in the raw bitumen to about 9% in the blown product fusing at 104° C. Blown bitumen fusing at 133°, 151° and 169° C. contained 8.4, 7.8 and 8.07% of resins respectively.

Although the hardening which takes place when Alberta bitumen is subjected to heat and air treatment may be initially ascribed to the decrease in the content of oily constituents and their conversion to asphaltenes of higher molecular weight, the later stages of this transformation to a material of high fusing point is no doubt due in large measure to the polymerization of the asphaltenes. Evidence in support of this view was obtained from data on the molecular weight of the oils, resins and asphaltenes extracted from the various bitumen samples. The oily constituents varied in molecular weight from 473 in the raw bitumen to 493–565 in the blown bitumen, the molecular weight decreasing with rise in fusing point. The molecular weight of the resins was 733 in the unblown bitumen, and in the blown samples it varied from 874 to 1012. The molecular weights of the asphaltenes were determined by Rast's method in camphor, since cryoscopic measurements in benzene gave high values similar to those obtained by Sakhanov. Furthermore, there was some separation of the asphaltenes from blown samples into two phases at the freezing point of benzene. For this reason the writer is of the opinion that the molecular weights of asphaltenes in benzene, as determined by the cryoscopic method, are probably much higher than the true values. The asphaltenes in the unblown bitumen had a mean molecular weight of 2219. This value was increased slightly to 2560 in the asphaltenes from bitumen blown to a fusing point of 104° C. Thereafter the molecular weight of the asphaltenes rose rapidly with increase in softening point of the bitumen, a mean value of 4690 being obtained in the fraction isolated from bitumen fusing at 169° C. The data in Table I indicate the extent of poly-

TABLE I

ASPHALTENES IN ALBERTA BITUMENS OF VARIOUS CONSISTENCIES

Description of bitumen	Fusing point (*R* and *B* method), ° C.	Asphaltenes, %	Molecular weight of asphaltene fraction	Softening points or sintering points of asphaltenes, ° C.
Original bitumen	34	24.2	2219	156–162
Blown bitumen	104	47.8	2560	255–263
Blown bitumen	133	48.4	3130	304–308
Blown bitumen	151	53.4	4225	355–340
Blown bitumen	169	57.8	4690	360–368

merization of the asphaltenes with rise in fusing point of the bitumen, as well as the relation between the softening or sintering points of the asphaltenes and their molecular weight. It is evident that the molecular size and percentage of asphaltenes present in blown asphalts exert a marked influence on their physical consistency.

Ultimate Composition of Blown Alberta Bitumen

The behavior of asphalts and asphalt base oils on oxidation is in marked contrast to that of paraffins. Whereas the latter are readily converted by oxidation into mixtures of fatty acids of about one-half the average number of carbon atoms in the molecule, involving the combination of considerable amounts of oxygen (7), this is not the case with asphalts and asphalt base oils which contain only small amounts of oxygen even after blowing for several days at temperatures of 200 to 275° C. The effect on Alberta bitumen of oxidation with air at 270 to 275° C. was similar to that on asphalts. As shown

TABLE II

Oxygen content of blown Alberta bitumen

Period of blowing (hr.)	0	4	6	8.5	14	—
Fusing point, ° C.	34	79	104	133	151	169
Oxygen in product, %	Nil	Nil	Trace	Trace	0.60	1.88

in Table II, only 0.6 % of oxygen was found in bitumen after blowing for 14 hr. at 270° C. at the rate of 6 cc. of air per minute per gram of material. After raising the softening point of the bitumen to 169° C., 1.88% of oxygen was found to have combined with the material.

The oxygen was distributed mainly in the resin fraction of the bitumen, and to a lesser extent in the asphaltenes. The oily constituents were free from oxygen.

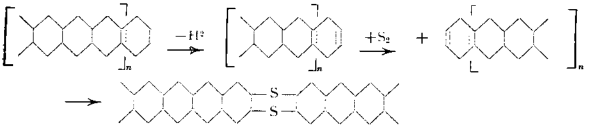
TABLE III

Ultimate composition of Alberta and commercial bitumens

Description of bitumen	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen, %
Alberta bitumen, f.p. 34° C.	85.43	9.52	4.52	0.57	Nil
Blown Alberta bitumen, f.p. 151° C.	84.4	9.17	5.23	0.60	0.6
Blown Alberta bitumen, f.p. 169° C.	83.32	8.99	5.25	0.56	1.88
Trinidad asphalt (15, p. 29)	82.33	10.69	6.16	0.81	—
Bermudez (15, p. 29)	82.88	10.79	5.87	0.75	—
Commercial grade blown asphalt, f.p. 149° C.	84.83	9.06	4.93	0.63	0.55
Commercial grade blown asphalt, f.p. 168° C.	85.16	8.98	3.51	1.07	1.28

It is of interest to compare the ultimate composition on an ash-free basis of the raw bitumen with that of the highly blown product and other natural and blown asphalts. The blown Alberta bitumen is quite similar in composition to the corresponding commercial grades of blown asphalt used as mineral rubbers. The main difference between the bitumen fusing at 169° C. and the commercial product fusing at 168° C. lies in the somewhat lower sulphur content of the latter. Throughout this study corresponding determinations of chemical composition and physical properties were made, for purposes of comparison on two well known commercial grades of bitumen used in the rubber industry (*c.f.* Table III).

The results obtained from the ultimate analysis of a series of blown bitumen products and their corresponding chemical components, *i.e.*, the oily constituents, resins and alphaltenes, indicate that condensation and polymerization are the essential transformations which take place during blowing or oxidation of asphalts at elevated temperatures. The oxygen of the air probably acts as a catalyst. A small amount of hydrogen (less than 0.5%) is liberated during the condensation of the oily constituents to asphaltenes, through the intermediate resin stage, and it combines with the oxygen to form water. Unsaturated compounds formed at this stage are rapidly polymerized to products of higher molecular weight. The substances formed are saturated and cyclic in nature, since unsaturation determinations with Waller's solution (21) on the oils, resins and asphaltenes yielded zero values in nearly every case. It appears therefore that polymerization takes place through the scission of the ring compounds in such a manner that a small fragment of the molecule, or two atoms of hydrogen, are split off and the remainder unites with a similar molecule. This may take place through the agency of sulphur and, to a lesser extent, oxygen. Sulphur evidently plays an essential part in the polymerization, since the resins contain about 6% and the asphaltenes 7% of sulphur. Even higher amounts of sulphur have been found in the asphaltenes from Trinidad asphalt (13). The greater part of the sulphur present in the original bitumen is held in combination in the asphaltene fraction. The mechanism of the polymerization may be represented as follows.



Some insight may be gained into the extremely complex nature of these substances by calculating the number of carbon atoms present in the molecule in the asphaltene fraction of Alberta bitumen. An average molecule in the asphaltenes from raw bitumen consists of approximately 140 carbon atoms, 168 hydrogen atoms and 4 sulphur atoms. In blown bitumen, the asphaltene molecule may consist of double this number of atoms.

Experimental

The Sample

The Alberta bitumen used in this work was part of a shipment made to the National Research Laboratories by Dr. K. A. Clark of the Research Council of Alberta. The material had been extracted from tar sand by Dr. Clark, according to methods developed by him (4). Before use, the bitumen was carefully dehydrated and found to contain only about 0.5% of water. The ash content of various samples was from 4.3 to 4.6%. Analysis of this ash indicated that it was a finely divided clay of the composition shown in Table IV.

TABLE IV

COMPOSITION OF ASH OF ALBERTA BITUMEN

SiO_2, %	Al_2O_3, %	Fe_2O_3, %	CaO, %	MgO, %	Total, %
56.1	25.0	16.1	1.6	0.9	99.7

The bitumen, after dehydration, possessed a fusing point of 34° C.

Apparatus

The apparatus used for blowing the bitumen with air is shown in Fig. 1. The sample was placed in an iron still-pot, *A*, equipped with a tightly fitting cover plate, a blow coil, *F*, a stirrer, and a thermometer well, *D*. A reflux condenser, *E*, fitted into the cover plate, the upper portion of the condenser being filled with glass beads. The still-pot was supported by a small tripod in an oil bath, *B*, containing an oil, of high flash point, known as Superheat valve oil. The bath was heated by gas burners and the temperature was controlled by means of the mercury regulator, *C*, operating on the gas feed line. The regulation of temperature was effected by the expansion and contraction of the air entrapped above the mercury column in the inverted bulb. Air was sent into the surge tank, *K*, and was measured with the wet-test meter, *H*, and the calibrated capillary flow meter, *G*, before passing through the bitumen.

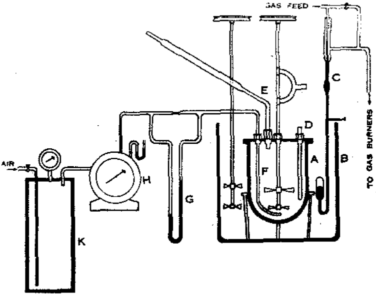


FIG. 1. *Apparatus for blowing bitumen.*

Effect of Air at 270 to 275° C.

Preliminary experiments without a reflux condenser indicated that from 25 to 45% of a yellow to reddish brown oil may be removed from the bitumen

by blowing with air at elevated temperatures. However, the residue w found to be very brittle, insoluble in rubber and lacking in ductility. C the other hand, products possessing a high dispersive power in rubber an readily soluble in solvents were prepared by condensing the greater part c the volatile oil formed during blowing and returning it as reflux to the con tents of the still. Some of the results have been presented in Table V. Th rate of blowing has been calculated on the basis of the number of cubic centimetres passed through per minute per gram of sample. The fusing point of the blown bitumen was determined by the ring and ball method according to A.S.T.M. specifications. At the temperature and rate of blowing employed, the fusing point of Alberta bitumen rises with increase in the time of blowing according to a linear relation, until a fusing point of 133° C. is reached. Beyond this stage the fusing point increases less rapidly with the time of blowing (Fig. 2). The fusing point may also be increased by either increasing the rate of blowing or raising the temperature. However it is impracticable to blow Alberta bitumen at temperatures above 285° C., since on several occasions when this was attempted a rapid evolution of gas took place with a sudden rise in the temperature of the bitumen. This reaction must be due to oxidation since it ceased when the air stream was turned off.

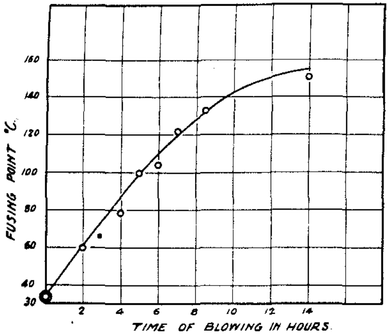


FIG. 2. *Increase of fusing point with time of blowing Alberta bitumen.*

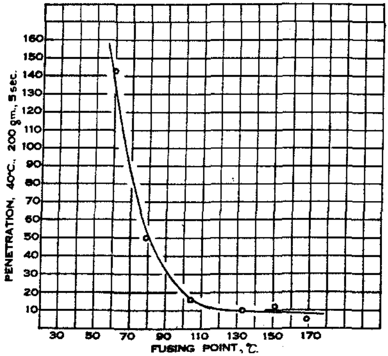


FIG. 3. ***Relation between fusing point and penetration of blown Alberta bitumens.***

The effect of air blowing on the penetration of Alberta bitumen is shown in Table VI. The unblown material is highly susceptible to change in temperature. **Air blowing reduces this susceptibility. The penetration at a given temperature**

decreases very rapidly with rise in fusing point in the early stages of blowing. Beyond a fusing point of 104° C. the effect on penetration is quite small (Fig. 3).

TABLE V

EFFECT OF BLOWING WITH AIR ON FUSING POINT OF ALBERTA BITUMEN

Sample no.	Weight of sample, gm.	Temperature of oil bath, ° C.	Rate of blowing, cc. of air per min. per gm. of sample	Period of blowing, hr.	Volatile oil distilled out, %	Fusing point of product, °C.
36	1500	270	6.0	2.0	3.0	60
35	1500	270	6.0	4.0	4.0	79
37	1500	270	6.0	5.0	5.7	100
39	1500	270	6.0	6.0	8.7	104
38	1500	270	6.0	7.0	11.2	122
40	1500	270	6.0	8.5	11.5	133
31	4670	270	6.0	14.0	6.5	151
33	4619	275	8.0	13.5	11.1	169
22	400	275	2.0	21.0	11.0	168
25	400	275	6.0	8.0	10.0	156
*26	400	275	6.0	9.0	9.5	150
†27	400	275	6.0	6.5	3.5	157
‡24	400	275	4.0	6.0	6.3	156

**This sample was blown in two stages of 5½ and 3½ hr., necessitating re-heating of the bitumen after the first stage to 275° C.*

†*Sample contains 15% Gilsonite.*

‡*Sample contains 1% anhydrous stannic chloride.*

TABLE VI

PENETRATION OF ALBERTA BITUMEN

Sample no.	Fusing point, ° C.	Penetration					
		40° C. 100 gm., 5 sec.	40° C. 200 gm., 5 sec.	25° C. 100 gm., 5 sec.	25° C. 200 gm., 5 sec.	5° C. 100 gm., 5 sec.	5° C. 200 gm., 5 sec.
Unblown	34	Too fluid	Too fluid	184.5	250.5	23.5	47.0
36	60	95.7	143.5	15.5	27.3		
35	79	21.3	49.7	6	10.2		
39	104	6.8	15.7	3	5.4		
40	133	2.2	9.7	Too viscous	0.0		
31	151	0.0	11.8	Too hard			
33	169	0.0	5.0	Too hard			
Hard Hydrocarbon	149	3.7	7.0	1.0	4.5		
Genasco	168	2.0	3.8	0.0	2.8		

Method of Separating the Components of Bitumen

The following procedure was adopted for the determination of asphaltous acids and anhydrides, since the method of Marcusson was found to lead to the formation of troublesome emulsions.

1. *Saponifiable matter.* About 5.00 gm. of substance was dissolved in 5(cc. of benzene by boiling under a reflux condenser. The solution was filtere(and 25 cc. of normal alcoholic potassium hydroxide added and the mixtur(refluxed for about 1½ hr. The mixture while still warm was transferred to a separatory funnel containing 150 to 200 cc. of 40% ethyl alcohol in hot water 25 cc. of a 5% solution of potassium chloride was added, and finally 100 cc. of benzene. The contents of the separatory funnel was agitated vigorously for several minutes and allowed to stand until separation took place. The benzene layer was removed and worked up as under Section 2. The alcoholic soap solution was drawn off and exhausted with small portions of benzene to remove unsaponifiable matter. The benzene layer was similarly exhausted with 40% ethyl alcohol to remove saponifiable matter. The alcoholic extracts were combined and the soap solution evaporated to a volume of about 100 cc. The asphaltous acids and anhydrides were liberated by the addition of dilute hydrochloric acid and then exhausted with benzene. The benzene extract was evaporated to a small bulk and the evaporation of the solvent completed at 100° C. The asphaltous acids and anhydrides were dried to constant weight. In appearance they were brown masses, the material from the raw bitumen and sample No. 39 being somewhat resinous and tacky, while that from the remaining samples was solid.

In several instances during the above determinations of saponifiable matter, an intermediate layer was formed in the separatory funnel, between the benzene and alcohol-water layers. This consisted of emulsified material which was rather difficult to break up by the further addition of either water or benzene. It was therefore evaporated to about 25 cc. and treated with 75–100 cc. of 40% aqueous alcohol, thus breaking the emulsion. The soap solution was exhausted with small amounts of benzene and then combined with the main alcoholic extract.

The asphaltenes, resins, and oily constituents were determined according to the methods of Marcusson (12).

2. *Asphaltenes.* After removing the saponified material the original benzene layer was evaporated to a volume of 25 cc. and the solution poured into 300 cc. of petrolic ether, b.p. 35°–60° C. The mixture was allowed to stand for several hours and the precipitated asphaltenes filtered. The precipitate was carefully washed with petrolic ether, dried at 100° C. and weighed. The filtrate and washings were combined and examined further.

Resins. The combined petrolic ether filtrates were evaporated to a volume of 25 to 30 cc. and then distributed over 50 gm. of fuller's earth or 100 gm. of silica gel. The oily constituents were removed by extraction of the material with petrolic ether, b.p. 35–60° C. The adsorbed resins were then removed by extraction with chloroform. The chloroform was distilled off and the resins dried at 100° C. and weighed.

Oily Constituents. The petrolic ether extract obtained as above was evaporated on the water bath and the solvent removed. The oil was finally kept at

a temperature of 100° C. for several hours to remove traces of solvent and then weighed. In cases where the first extract was dark in color, it was concentrated to 25 cc. and again treated with fuller's earth.

The components of natural and blown Alberta bitumen were separated according to this procedure and compared with two blown asphalts which are widely used in the rubber industry as mineral rubbers. The data are presented in Table VII.

TABLE VII

COMPONENTS OF NATURAL AND BLOWN ALBERTA BITUMEN

Bitumen	Asphaltous acids and anhydrides, %	Asphaltenes, %	Resins, %	Oily constituents, %	Ash, %
Raw bitumen	1.85	24.2	15.0	55.0	4.3
Bitumen, f.p. 104° C.	0.87	47.8	9.3	37.8	4.5
Bitumen, f.p. 133° C.	0.64	48.4	8.4	39.2	4.2
Bitumen, f.p. 151° C.	0.46	53.4	7.8	34.9	4.62
Bitumen, f.p. 169° C.	0.42	57.8	8.07	29.1	4.38
Hard Hydrocarbon, 149° C.	0.25	45.9	13.57	40.2	0.21
Genasco, f.p. 167° C.	0.45	47.4	12.2	39.8	0.51

The amounts of asphaltous acids and anhydrides decrease with rise in fusing point of the bitumen from an original value of 1.85 to 0.42% in the material of highest fusing point. The asphaltenes show a progressive increase in amount with rise in fusing point of the bitumen. The amount of resins, on the other hand, decreases from an initial value of 15.0% to about 8% in the material fusing in the temperature range 133°–169° C. The percentage of oily constituents decreases from 55.0 in the unblown bitumen to 29.1 in that with a softening point of 169° C. Both the Hard Hydrocarbon and Genasco mineral rubbers contain a higher amount of oily constituents and a lower amount of asphaltenes than comparable samples from blown Alberta bitumen. The asphaltenes from the two commercial grades of mineral rubber, on the other hand, soften at much higher temperatures and possess higher molecular weights than those extracted from corresponding grades of blown bitumen.

The asphaltenes from the different Alberta bitumen samples form a well defined series, the lowest number of which softens over the temperature range 156–162° C. and the highest at 360–368° C. as shown in Table VIII. The sintering points were obtained by means of an electrical melting point apparatus, at least three determinations being carried out on each sample.

TABLE VIII

RANGE OF SINTERING POINTS OF ASPHALTENES

Asphaltenes precipitated from:—	Softening or sintering point range, ° C.	Remarks
Raw bitumen, f.p. 34° C.	156–162	
Bitumen, f.p. 104° C.	255–263	Cracking began at 276–288° C. Brown oil distilled off.
Bitumen, f.p. 133° C.	304–308	Cracking set in at 312° C. Brownish oil distilled off.
Bitumen, f.p. 151° C.	335–340	Brownish oil began to distil at 340–345° C.
Bitumen, f.p. 169° C.	360–368	Reddish brown oil given off at this temperature.
Hard Hydrocarbon, f.p. 149° C.	387–395	Cracking set in at about 395–405° C. with evolution of reddish brown oil.
Genasco, f.p. 167° C.	385–395° C. Melts rapidly at 395° C.	Cracking set in at 395–405° C. with evolution of reddish brown oil.

TABLE IX

IODINE NUMBER OF BITUMEN COMPONENTS

Sample	Iodine no.
Resin from natural Alberta bitumen	6.2
Asphaltenes from Hard Hydrocarbon	1.1
Asphaltenes from Genasco	19.5

Iodine Number

Unsaturation determinations were carried out on the oil, resin and asphaltene fractions listed in Table VII, using Waller's solution. Zero values were obtained for the iodine number in all cases except those shown in Table IX.

Properties of Oils, Resins and Asphaltenes

The oils from the natural and blown Alberta bitumen were yellow to light red, while those from Hard Hydrocarbon and Genasco were darker. The refractive indices of the oils are shown in Table X. The refractive index of the oils from the Hard Hydrocarbon and Genasco could not be measured accurately because of their color.

TABLE X

REFRACTIVE INDICES OF OILS FROM NATURAL AND BLOWN ALBERTA BITUMEN

Oil from:	n^{25} ° C.
Raw bitumen	1.4999 (d^{25} = 0.940)
Bitumen No. 39	1.4986
Bitumen No. 40	1.4982
Bitumen No. 31	1.4975
Bitumen No. 33	1.4964

The resins from raw Alberta bitumen are soft and tacky and reddish brown. Those from blown bitumen are solid, melt at about 80° to 90° C. and are also red-brown. They are readily soluble in benzene, chloroform, carbon tetrachloride and petrolic ether, sparingly soluble in hot acetone and insoluble in alcohol. The resins form true solutions and impart an intense coloration, from yellow to red, to these solutions, depending on the concentration. This

color is more intense in the resins of higher molecular weight. The specific gravity of the resins from raw Alberta bitumen is 1.002 at 27° C.

The asphaltenes partake of the nature of hemicolloids. They are readily soluble in benzene, carbon disulphide, chloroform, carbon tetrachloride, but insoluble in petrolic ether, acetone or alcohol. The solutions when dilute, are deep red and in more concentrated solutions, reddish-brown. The coloring power of the asphaltenes is much greater than that of the resins. The solubility of the asphaltenes in benzene decreases with rise in molecular weight. They are apparently insoluble in the rubber hydrocarbon, since solutions of rubber and asphaltenes in benzene when mixed show a separation of the asphaltenes.

This behavior indicates that these two polymeric materials differ basically in structure, the rubber hydrocarbon being essentially built up of long chains, aliphatic in nature, whereas the asphaltenes probably possess a highly developed polycyclic structure.

The density of asphaltenes from raw bitumen at 27° C. is 1.126.

The writer has separated the oily constituents, resins, and asphaltenes from two kilograms of Alberta bitumen using a large copper Soxhlet extractor. A further examination of the properties of these materials will form the subject of a separate paper.

Molecular Weights of the Oils, Resins and Asphaltenes

The molecular weights of the oils and resins were determined quite readily by the cryoscopic method in benzene. Before determining the molecular weight of the asphaltenes, they were reprecipitated from benzene solution with petrolic ether. Cryoscopic measurements on solutions of asphaltenes in benzene yielded values between 4300 and 5600. The results were not consistent however and on closer examination it was found that a slight separation into two phases took place at the freezing point of the solution.

The asphaltenes were found to yield homogeneous solutions in camphor and the molecular weight of these fractions were therefore carried out according to the method of Rast. The results are presented in Table XI.

TABLE XI

MOLECULAR WEIGHTS OF OILS, RESINS AND ASPHALTENES (DERIVED BY EXTRACTION FROM BLOWN BITUMEN)

Origin of fraction	Mol. wt. of oily constituents (Cryoscopically in benzene)	Mol. wt. of resins (Cryoscopically in benzene)	Mol. wt. of asphaltenes (Rast)	Origin of fraction	Mol. wt. of oily constituents (Cryoscopically in benzene)	Mol. wt. of resins (Cryoscopically in benzene)	Mol. wt. of asphaltenes (Rast)
Raw bitumen, f.p. 34° C.	473	733	2219	Bitumen, f.p. 169° C.	493	930	4690
Bitumen, f.p. 104° C.	560	874	2560	Hard Hydrocarbon, f.p. 149° C.	700	1497	4870
Bitumen, f.p. 133° C.	565	956	3130	Genasco, f.p. 168° C.	684	1410	5160
Bitumen, f.p. 151° C.	497	1012	4225				

The molecular weights of the oils in bitumen increase from 473 in the raw bitumen to 565 in bitumen fusing at 133° C., followed by a drop to 493 in bitumen fusing at 169° C. The oils from Hard Hydrocarbon and Genasco are of considerably higher molecular weight than those taken from analogous Alberta bitumen samples. The average molecular weights of the resins increase from 733 to 1012 owing to polymerization. The resins from the two commercial samples are of considerably higher molecular weight as in the case of the oils.

The asphaltenes are gradually polymerized by the air blowing treatment accorded the bitumen at 270 to 275° C. The average molecular weight of the lowest fraction is 2219 and this value increases with rise in fusing point of the blown bitumen to an average value of 4690 in the asphaltene fraction from the sample fusing at 169° C. The sintering points of the asphaltenes increase with rise in molecular weight in a manner which suggests that these snbstances form a series of complex polymers (Table VIII).

Ultimate Analysis of Blown Bitumen, Oily Constituents, Resins and Asphaltenes

A series of samples of blown bitumen, and the oily constituents, resins and asphaltenes, were subjected to full elementary analysis in order to determine the effect of the air and heat treatment. Determinations were made of carbon, hydrogen, sulphur and nitrogen. Carbon and hydrogen were determined in an electrical combustion apparatus. The sulphur was first determined by fusion with sodium peroxide in nickel crucibles according to the A.O.A.C. official method. This was found to check with the Parr bomb method in the case of oils and resins. The blown bitumen samples and the asphaltene fractions, on the other hand, yielded better results when the Parr bomb was used for carrying out the fusions than when the A.O.A.C. open crucible method was used. In the latter case the results were too low owing to difficulty in carrying out a complete fusion. All the solid samples were finely ground in a mortar, prior to analysis, but in those cases where the material was too plastic to be ground, it was found expedient to dissolve the sample in benzene free from sulphur, mix with a little sodium carbonate, dry at 90° C. and then grind and mix with the proper amount of fine sodium peroxide in the Parr bomb.

The nitrogen was determined by the Kjeldahl method.

In comparing the data presented in Table XII, it must be borne in mind that raw Alberta bitumen is not a material of fixed composition; considerable variations may occur in samples extracted from Alberta sands from different localities. What is probably an extreme case of variation in composition may be shown by comparing the data in the table on raw bitumen with the values obtained by Krieble and Seyer (8) on their sample extracted from sand taken from an exposure on the Hangingstone river, three miles from Fort McMurray. Their values for the raw bitumen are:—C, 84.49; H, 11.23; S, 2.73; and N, 0.04%. Nearly all the samples analyzed by Dr. Clark of the Research Council of Alberta contain between 4 and 5% of sulphur (3).

TABLE XII

ELEMENTARY COMPOSITION OF BLOWN BITUMEN (ASH-FREE BASIS)

Description of sample	Fusing point, ° C.	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference	Total
Raw bitumen	34	85.43	9.52	4.52	0.57	Nil	100.04
Bitumen No. 36	60	84.71	9.71	5.30	0.56	Nil	100.28
Bitumen No. 35	79	84.65	9.54	5.34	0.56	Nil	100.09
Bitumen No. 37	100	84.90	9.13	5.34	0.62	Nil	99.99
Bitumen No. 39	104	84.50	8.88	5.42	0.73	Trace	99.53
Bitumen No. 38	122	85.27	8.87	5.37	0.65	Trace	100.16
Bitumen No. 40	133	84.60	9.05	5.25	0.65	Trace	99.55
Bitumen No. 31	151	84.4	9.17	5.23	0.60	0.6	100.0
Bitumen No. 33	169	83.32	8.99	5.25	0.56	1.88	100.0
Hard Hydrocarbon	149	84.83	9.06	4.93	0.63	0.55	100.0
Genasco	168	85.16	8.98	3.51	1.07	1.28	100.0

The samples in Table XII were prepared from the same batch of raw material contained in a shipment made by Dr. Clark, the variation in elementary composition of which may be assumed to be quite small. As the melting point of the bitumen rises with continued blowing, there occurs a drop in the carbon and hydrogen content. The sulphur and nitrogen content appear to be unaffected by this treatment. The net result is a gradual combination with oxygen. This oxidation of the asphalt is appreciable when the melting point is about 150° C. or higher. The over-all experimental error involved in the analyses is about $\pm 0.3\%$ and it is therefore difficult to detect the presence of oxygen in amounts below 0.5%. However it would appear from the analyses of the resins (Table XIV) that the bitumen melting at 104° C. contains only a small amount of oxygen. The resins from this sample contain 1.4% of oxygen. No oxygen appeared to be present in the oil and asphaltene fractions. Since the resin fraction was 9.3% of the bitumen, the total oxygen in this sample is about 0.13%. The hydrogen removed from the bitumen during blowing is converted in part into water by combination with oxygen from the air. Some of the hydrogen however is used up in enriching the oil formed during prolonged blowing when the melting point of the bitumen

TABLE XIII

ELEMENTARY COMPOSITION OF OILY CONSTITUENTS

Description of sample	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %
Oily constituents from raw bitumen	86.99	11.02	2.08	0.28
Oily constituents from bitumen No. 39	86.46	10.49	3.60	0.18
Oily constituents from bitumen No. 40	85.75	10.63	3.76	0.12
Oily constituents from bitumen No. 31	86.46	11.10	2.74	0.10
Oily constituents from bitumen No. 33	86.36	11.02	2.69	0.06
Oily constituents from Genasco	86.76	10.70	2.46	0.15
Oily constituents from Hard Hydrocarbon	85.30	10.60	4.30	0.11

rises above 133° C., since the hydrogen content of the oily constituents shows an increase in samples 31 and 33 (Table XIII).

It is of interest to observe that the bitumen with a fusing point of 151° C. agrees very closely in elementary composition with Hard Hydrocarbon, a popular grade of mineral rubber having a similar fusing point. The elementary composition of Genasco, another commercial grade of mineral rubber, differs from its analogue, sample No. 33, in having a lower sulphur content but a higher nitrogen content (Table XII).

A comparison of the data in Tables XIII, XIV and XV, shows the distribution of sulphur, nitrogen, and oxygen in the various components which make up the complex material known as bitumen, *i.e.*, the oils, resins and asphaltenes. The largest portions of the sulphur and nitrogen are present in the asphaltene fractions, with correspondingly smaller amounts in the resins and oils. The oils are free from oxygen, whereas the resins of the blown samples contain above 1% of oxygen, increasing to 2.65% in the most highly blown material. In the case of the asphaltenes, only those fractions extracted from bitumen fusing above 133° C. contain oxygen. The oxygen content then increases with rise in melting point.

TABLE XIV

ELEMENTARY COMPOSITION OF RESINS

Description of sample	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference
Resin from raw bitumen	84.28	9.39	6.02	0.40	Nil
Resin from bitumen No. 39	82.62	9.66	5.62	0.64	1.4
Resin from bitumen No. 40	83.14	9.24	6.01	0.56	1.05
Resin from bitumen No. 31	82.81	9.11	5.67	0.58	1.83
Resin from bitumen No. 33	81.35	9.63	5.87	0.50	2.65
Resin from Hard Hydrocarbon	82.68	8.88	6.70	0.65	1.09
Resin from Genasco	83.12	9.75	4.72	0.89	1.52

TABLE XV

ELEMENTARY COMPOSITION OF ASPHALTENES

Description of sample asphaltenes from:—	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference	Total
Raw bitumen	84.0	8.42	6.58	0.99	Nil	99.99
Bitumen No. 39	83.93	7.93	7.08	1.26	Nil	100.20
Bitumen No. 40	84.11	7.85	6.98	0.96	Nil	99.90
Bitumen No. 31	83.81	7.84	6.94	0.98	0.43	100.0
Bitumen No. 33	82.34	7.76	6.72	1.02	2.16	100.0
Hard Hydrocarbon	84.56	7.81	5.07	0.99	1.57	100.0
Genasco	86.31	7.61	3.24	1.41	1.43	100.0

Both the carbon and hydrogen content of the oils (Table XIII) show a decrease in the early stages of blowing, *i.e.*, polymerization of the oil occurs due to condensation reactions involving the removal of hydrogen, followed

by an increase in the later stages due probably to "cracking" reactions and the formation of products of lower molecular weight. The molecular weights of the oils, as already pointed out, increase from 473 in the raw bitumen to 565 in bitumen fusing at 133° C. followed by a drop to 493 in the sample of highest fusing point.

The essential differences in the composition of the resins compared with the oils are the marked increase in sulphur content and the increasing amount of oxygen present. The resins appear to be quite susceptible to oxidation. Their high sulphur content is no doubt due to the fact that polymerization of the oils to the resin stage has been accomplished in part through the agency of sulphur. The nitrogen content of the resins is appreciably higher thar that of the oils.

The asphaltenes do not appear to be as susceptible to oxidation by blowing with air as the resins. They contain higher amounts of sulphur and nitrogen than the corresponding resin fractions from which they appear to be derived. These elements seem to be in a very stable form of combination since treatment with air for periods up to 14 hr. at 275° C. does not alter to any appreciable extent the proportions present in the asphaltenes. The net result of such treatment is a gradual polymerization of the asphaltenes from a molecular weight of 2219 in the raw bitumen fraction to 4690 in that taken from blown bitumen fusing at 169 ° C.

Acknowledgments

The author wishes to thank Mr. T. R. Griffith for assistance in connection with the blowing experiments on bitumen, and Mr. C. W. Davis for assistance in carrying out the sulphur analyses.

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HEAT CAPACITY MEASUREMENTS ON GELATIN GELS[1]. II.

By W. F. Hampton[2] and J. H. Mennie[3]

Abstract

e u ements of the heat capacity of gelatin gels, prepared from ash-free, electric gelatin, have been extended to cover the temperature range between −180° and 25° C. and the concentration range from 9 to 100% gelatin. Change in the temperature of the gel above the freezing point is found to be accompanied by a heat effect which has not previously been taken into account in calorimetric measurements on gels and which renders the interpretation of the experimental data uncertain.

Introduction

In a previous paper (3) a method for determining the heat capacity of gelatin gels was described and results reported for a 24% gel, dry gelatin and a gel containing 12.5% water. The calculation of unfrozen or "bound" water from such measurements was discussed and a new equation was derived. In the present paper further investigations are reported covering the range of concentration of gelatin gels from 9 to 100% gelatin, over the temperature range from −180° to 25° C.

Experimental

Apparatus

The apparatus and procedure have already been described (3, 4). Only slight modifications were necessary to make the method applicable over the wider range of concentration and of temperature.

The gelatin used was the same "ash-free", isoelectric gelatin supplied by the Eastman Kodak Co. (Lot No. 48). The ash content was 0.04% and the pH was given as 4.85.

The gels were prepared as before by soaking the sheets of gelatin in distilled water, which had been boiled and allowed to cool to room temperature, long enough for the gelatin to take up approximately the desired amount of water. In the case of the more dilute gels the water-soaked material was heated at 50° C. for about 30 min. and finally poured into the container. The container was then rotated in a horizontal position at about 300 r.p.m. until the gel had set. This caused the gel to set around the sides of the container, giving improved heat transfer and also leaving a cylindrical space in the centre of the gel which permitted expansion on freezing.

This method of filling the container was found to be very satisfactory for gels up to about 45% gelatin. The very high viscosity in the sol form of more concentrated gels prevents pouring them readily. Hence, in the case of gels containing more than 45% gelatin, the container was filled by pressing the water-soaked material into it, and the heating at 50° C. was carried out with the sample enclosed in the container. Since in gels of these higher con-

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centrations there is very little expansion during freezing, the procedure of rotating the container as the gel cooled was omitted. The percentage of dry gelatin in the concentrated gels was obtained by cutting portions from various parts of the water-soaked sheets of gelatin immediately they were removed from the water. These were analyzed by heating to constant weight in an oven at 105° C. In all cases duplicate determinations were made and the average taken as the concentration of the gel.

As before, an ice-water mixture was used as the constant temperature bath at 0° C. and a solid carbon dioxide—ether mixture for −78.5° C. Temperatures between these limits were obtained by cooling ether or acetone to the desired temperature and maintaining it at a constant value by dropping in small lumps of solid carbon dioxide at regular intervals. Liquid air was used to give the lowest temperatures, near −183° C. The temperature was always carefully checked by means of the platinum thermometer, since the boiling point of liquid air varies with the nitrogen content.

The arrangement of the bath for temperatures near −130° C. is illustrated in Fig. 1. The bath liquid was obtained by distilling gas machine naphtha and collecting the fraction boiling below 30° C. This was brought to the desired temperature and maintained at this point by blowing liquid air at regular intervals into a very thin copper tube, *P*, suspended in the bath liquid. To make the cooling as efficient and rapid as possible, horizontal disks of copper were soldered to the copper tube, giving a set of vanes projecting into the bath liquid. Conduction of heat into the bath through the copper tube was minimized by fixing it to a short length of Pyrex tubing. This was done by painting the surface of the glass with gold paint, heating to attach the gold firmly to the glass, and depositing electrolytically a layer of copper on the gold. The copper tube was then soldered to the layer of copper on the glass. This arrangement was suspended in the bath liquid so that only the glass tubing projected above the surface. Stirring was effected by means of dry air and the temperature was measured by the calibrated platinum thermometer, *T*.

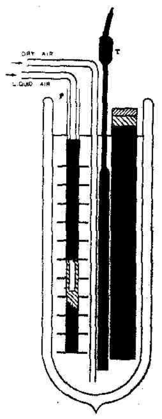

FIG. 1. *Bath for temperatures between −78.5° C. and −180° C.*

It is estimated that with careful manipulation the temperature of the thermostat could be controlled to about 0.2° at − 130° C. and easily to 0.1° at temperatures above −78.5° C.

Results

All heat capacity calculations were made to 25° C. The experimental values for the total heat capacity of container plus gel are given in Table I. The values for the 24% gel and those for the 87.5% gel and dry gelatin down to −78.5° C., which were reported in the previous paper (3), are here repeated

TABLE I

TOTAL HEAT CAPACITY OF CONTAINER PLUS GEL, IN CALORIES

Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity
Container alone		Dry gelatin, 7.6590 gm.		87.5% Gel, 9.7415 gm.		67.7% Gel, 10.0525 gm.		61.4% Gel, 8.4173 gm.	
25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0
0.0	−335.6	0.0	−389.5	0.0	−414.4	0.0	−501.4	0.0	−459.3
−26.7	−686.4	−20.2	−687.0	−29.2	−900.1	−4.9	−587.5	−2.1	−493.6
−57.8	−1077.9	−34.9	−909.4	−30.5	−905.8	−5.2	−599.5	−4.0	−533.6
−78.5	−1339.5	−56.2	−1207.2	−52.4	−1238.6	−9.2	−671.0	−5.0	−571.6
−132.6	−1925.5	−78.5	−1523.8	−78.5	−1613.6	−9.4	−672.0	−9.4	−672.3
−184.2	−2371.7	−131.6	−2166.6	−129.6	−2263.2	−23.3	−928.5	−15.0	−780.8
		−184.5	−2678.0	−183.5	−2781.6	−39.5	−1221.5	−32.8	−1075.1
						−78.5	−1849.9	−55.4	−1437.8
						−123.6	−2467.4	−78.5	−1798.7
						−184.1	−3113.0	−130.4	−2494.9
								−183.9	−3041.1
58.1% Gel, 9.1576 gm.		45.3% Gel, 15.1890 gm.		24.0% Gel, 12.2845 gm.		20.9% Gel, 11.9608 gm.		9% Gel, 12.9730 gm.	
25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0
0.0	−482.3	0.0	−636.9	0.0	−607.0	0.0	−604.3	0.0	−647.3
−2.2	−526.3	−1.1	−705.5	−0.8	−1087.4	−1.2	−1113.0	−1.0	−1471.6
−3.0	−544.1	−3.2	−902.2	−1.3	−1129.1	−5.0	−1306.5	−4.8	−1600.0
−5.0	−596.0	−6.1	−1062.8	−5.3	−1293.6	−10.7	−1438.8	−10.0	−1712.5
−8.5	−694.5	−11.6	−1212.4	−15.2	−1516.7	−22.6	−1668.1	−18.0	−1874.2
−12.0	−776.3	−25.6	−1510.9	−27.9	−1760.0	−51.7	−2183.8	−28.9	−2079.9
−25.6	−1011.4	−36.3	−1728.7	−38.3	−1941.5	−78.5	−2651.5	−52.6	−2514.8
−26.1	−1022.7	−45.0	−1884.9	−44.5	−2062.7	−134.1	−3455.4	−78.5	−2968.5
−47.4	−1379.6	−53.9	−2047.1	−54.2	−2234.2	−191.2	−4068.8	−124.8	−3673.2
−78.5	−1882.8	−78.5	−2490.5	−63.8	−2397.5			−193.2	−4441.8
−127.0	−2540.7	−123.6	−3213.8	−78.5	−2645.6				
−184.3	−3128.0	−184.7	−3926.1						

TABLE II

HEAT CAPACITY PER GRAM OF GEL, IN CALORIES

Temp., °C.	Dry gelatin	Gel							
		87.5%	67.7%	61.4%	58.1%	45.3%	24.0%	20.9%	9.0%
25.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.0	−7.12	−8.15	−15.92	−14.77	−16.08	−19.88	−22.09	−22.51	−24.05
−0.6	—	—	—	—	—	—	−40.40	—	—
−0.6	—	—	—	—	—	—	−54.14	—	—
−1.0	—	—	—	—	—	−22.65	−61.54	—	−86.64
−1.2	—	—	—	—	—	—	—	−63.56	—
−2.0	—	—	—	—	—	−27.78	—	−68.22	−88.96
−3.0	—	—	—	−16.16	−18.45	−33.38	−68.54	−71.48	−90.65
−5.0	—	—	—	−20.20	−21.18	−40.42	−71.88	−75.66	−92.73
−10.0	−9.27	−11.29	−21.39	−25.66	−29.16	−46.68	−76.38	−80.18	−95.97
−20.0	—	—	—	−31.36	−34.73	−52.34	−82.50	−85.36	−101.3
−40.0	−15.67	−19.81	−37.00	−39.92	−43.35	−61.49	−91.49	−93.80	−110.0
−60.0	—	—	—	—	—	—	−99.84	—	—
−78.5	—	—	—	—	—	—	−106.3	—	—
−80.0	−24.42	−28.54	−51.53	−55.36	−60.28	−76.50	—	−110.4	−126.3
−120.0	−30.94	−36.03	−61.87	−67.95	−71.74	−89.60	—	−123.0	−139.2
−180.0	−39.17	−42.29	−73.31	−79.24	−81.90	−101.40	—	−135.4	−151.5

for the sake of completeness. At the top of each column is given the concentration of the gel and the weight of the sample used. Values for the heat capacity of the container alone are also included. The values for the 24% gel are the average of at least two determinations each. All the values at 0° C. are also the average of two or more determinations. In other cases, experiments were repeated only when the behavior of the apparatus was erratic and gave reason to doubt the accuracy of the result, or when the transfer of the container from the constant temperature bath to the calorimeter was not successful. These experimental values were all plotted and gave smooth curves as illustrated in Fig. 2. The curves for the 24% and the 67% gels have been omitted from the figure to avoid confusion.

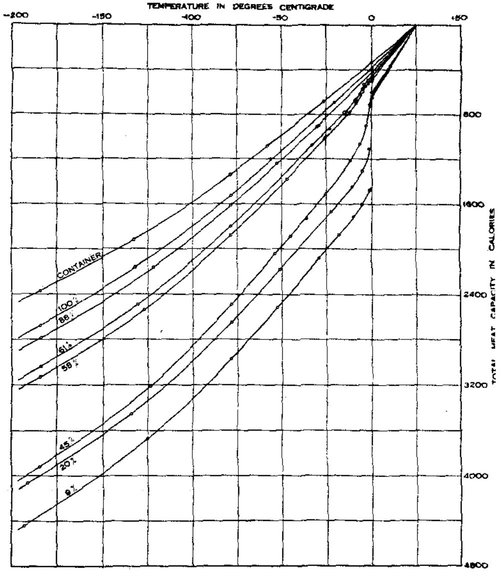

FIG. 2. *Experimental heat capacity curves for container plus gel.*

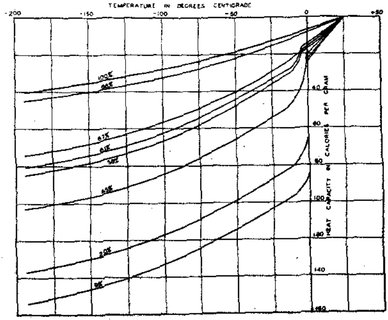

FIG. 3. *Heat capacity curves for gelatin gels.*

From these curves the values for the heat capacity of each of the gels at different temperatures were obtained by subtracting the value for the container at the same temperature. The weight of the sample of gel being known in each case, the heat capacity per gram of gel at different temperatures was calculated. These calculated values are given in Table II and are plotted (omitting the 24% gel) in Fig. 3. All values used in subsequent calculations were read from these smooth curves.

Discussion of Results

Freezing in Gels

From the general appearance of the heat capacity curves in Fig. 3, it is apparent that freezing certainly occurs in gels up to and including 61% gelatin. This is indicated by the definite break in the heat capacity curve which occurs a little below 0° C. The absence of such a break in the curves for the 67% and 87.5% gels apparently means that in these cases there is no freezing at any temperature.

The sharp breaks near 0° C. in the curves for the more dilute gels are evidently due to the latent heat of fusion of the water that is frozen in these gels. In all cases up to 61% gelatin the direction of the curve changes very rapidly between about −1° C. and −25° C. Evidently there is a gradual freezing of water taking place over this range of temperature. The curves are similar to those which would be obtained with a solution of a crystalloid of concentration less than that of the eutectic mixture, over the range between the freezing point and the eutectic temperature (7). However, it cannot be inferred that the mechanism of the freezing point lowering is the same in the two cases.

In the gel, the water is apparently held by forces which vary in intensity, so that the amount of the water which is frozen at a given temperature depends on how much of it can be freed from these forces at that temperature (5). It is well known that when gelatin swells in water there is an enormous contraction in the total volume of gelatin and imbibed water (10). To cause such a contraction in the volume of the water alone would require a tremendous pressure. It is possible therefore, that a portion of the water in a gel is acted upon by forces which are analogous to pressure forces. That water which is under greatest "pressure" would freeze only at the lower temperatures, and the temperature at which any portion of the water could be frozen would be dependent on the pressure acting upon it.

The behavior of the water on freezing these gels is shown more clearly by calculating the heat capacity at various temperatures of the water associated with one gram of dry gelatin. This is done by multiplying the figures read from the smooth curves of Fig. 3 by the weight of gel which contains one gram of dry gelatin, and then subtracting the heat capacity of the dry gelatin. The results are shown in Table III and are represented graphically in Fig. 4 in the form of a family of isothermals, the weight of the water associated with one gram of gelatin being plotted against its heat capacity.

TABLE III

HEAT CAPACITY TO 25° C. OF WATER ASSOCIATED WITH ONE GRAM OF GELATIN

% Gel	Gm. water	0° C.	−10° C.	−30° C.	−50° C.	−100° C.	−180° C.
9.0	10.11	260.7	1058.0	1165.2	1257.0	1449.4	1650.2
20.9	3.79	101.2	374.8	418.8	455.8	532.0	609.4
24.0	3.18	85.9	310.4	351.5	382.5	—	—
45.3	1.21	37.4	94.2	111.9	126.4	155.0	186.7
58.1	0.72	21.5	41.2	54.3	64.7	84.4	103.9
61.4	0.63	17.5	32.9	45.2	54.5	72.5	90.4
67.7	0.48	16.6	22.8	34.0	42.6	56.3	70.1
87.5	0.14	2.9	4.0	5.8	6.9	8.6	10.3

Beyond 0.6 gm. water per gm. dry gelatin, the isothermals are straight lines. The slope of each line gives the heat capacity of the "free" water in the gel between the corresponding temperature and 25° C. The slopes correspond very well with the measured heat capacity per gram of ice (including latent heat of fusion) as shown in Table IV. The figures for the heat capacity of ice were read from the smooth curve obtained by plotting the measurements of Barnes and Maass (1). These go only as low as −78.5° C. and the curve was extended to −180° C. by employing the results of Maass and Waldbauer (6) which agree exactly with those of Barnes and Maass at the higher temperatures.

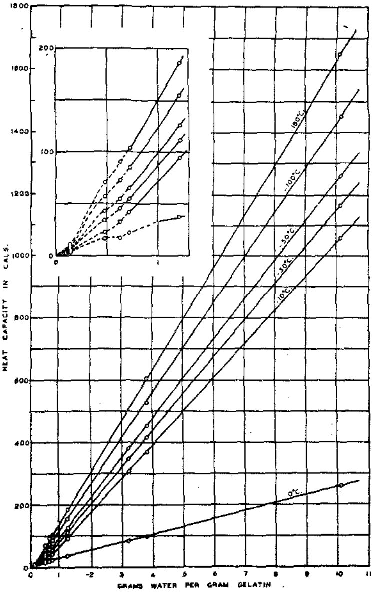

FIG. 4. *Isothermals for heat capacity of water associated with one gram of dry gelatin in gels of various concentrations.*

The point at which the isothermals deviate from the straight line should indicate the amount of water which is regarded as "bound", *i.e.*, which does not display the same heat capacity as "free" water. This is approximately 0.6 gm. per gm. dry gelatin but the data obtained do not admit of defining more closely than this the point where the line begins to bend.

TABLE IV

COMPARISON OF SLOPES OF ISOTHERMALS (FIG. 4) WITH HEAT CAPACITY OF ICE

Temp., °C.	0	−10	−30	−50	−100	−180
Slope of isothermal	*25.23*	*108.3*	*118.3*	*127.0*	*145.3*	*164.5*
Heat capacity of ice, cal.	25.05	109.3	118.6	127.3	146.2	166.3

The results are displayed a little differently in Table V and in Fig. 5, where the heat capacity per gram of the water in a gel is plotted against the weight of gelatin associated with one gram of water. This gives another set of isothermals.

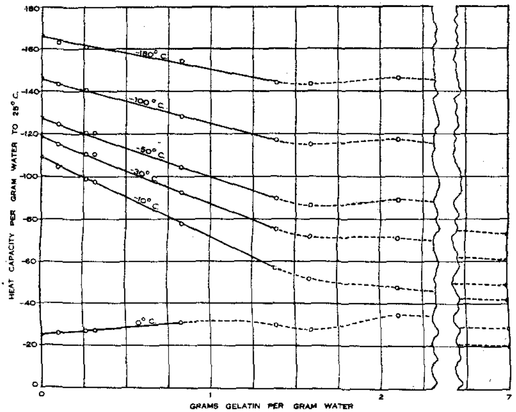

FIG. 5. *Isothermals for heat capacity per gram of the water in gels of various concentrations.*

TABLE V

HEAT CAPACITY PER GRAM TO 25° C. OF WATER IN GELATIN GELS

% Gel	Gm. gelatin per gm. water	0° C.	−10° C.	−30° C.	−50° C.	−100° C.	−180° C.
0.0	0.0	25.05	109.3	118.6	127.3	146.2	166.3
9.0	0.099	25.8	104.6	115.2	124.4	143.4	163.1
20.9	0.264	26.7	99.0	110.5	120.4	140.6	161.1
24.0	0.316	27.0	97.7	110.6	120.4	—	—
45.3	0.828	31.0	77.9	92.6	104.5	128.2	154.4
58.1	1.39	29.9	57.0	75.0	89.8	117.2	144.2
61.4	1.59	27.8	52.0	71.9	86.5	115.2	143.6
67.7	2.11	34.7	47.7	71.1	89.1	117.6	146.4
87.5	7.00	20.1	28.3	41.8	48.8	60.8	72.8

If all the water in the gels were "free" water, its heat capacity would not be affected by the presence of the gelatin and these isothermals would be horizontal straight lines, the height of each above the axis being given by the heat capacity of ice (or water) at the corresponding temperature. The downward slope of the actual isothermals, for temperatures below the freezing point, is due to the decreased heat capacity of the bound portion of the water, which remains unfrozen. They appear to be straight lines up to 1.39 gm. of gelatin per gm. of water (58.1% gel). That is, up to this concentration the decrease in heat capacity caused by adding gelatin to the water is directly proportional to the amount of gelatin added. The inference would seem to be that the amount of bound water is also directly proportional to the amount of gelatin added or, in other words, the amount of water bound per gram of gelatin is independent of the gel concentration up to the limit stated. The same inference may be drawn from the agreement already mentioned between the slopes of the straight lines in Fig. 4 and the heat capacity of ice.

The 0° isothermal of Fig. 5 is discussed below.

Calculation of "Bound" Water

In the previous paper (3) a method of calculating unfrozen or "bound" water was derived. It was based on the following equation,

$$H_{obs.} = aH_g + xH_x + (1-a-x)H_i - \lambda \ ,$$

where a represents the amount of dry gelatin and x the amount of bound water per gram of gel; $H_{obs.}$ is the measured heat capacity of the gel and H_g, H_x, and H_i the heat capacity per gram of dry gelatin, of bound water and of ice, respectively. The quantity λ was introduced to take into account any evolution of heat which might occur when the frozen portion of the water is reabsorbed into the gel after it has melted. On the basis of Rosenbohm's measurements (12) on the heat of swelling of gelatin, it was assumed that if the unfrozen portion of the water was not less than 0.5 gm. per gm. of dry gelatin (7, 8, 9), λ could be neglected.

H_g, the heat capacity of the dry gelatin, is obtainable from the data in Table II. Values of H_i are given by Barnes and Maass (1) down to −78.5° C. and by Maass and Waldbauer (6) to −180° C. It was thought that an estimate of H_x, the average heat capacity of the bound water, could be obtained from measurements on a gel which showed no break in the heat capacity curve and thus in which, presumably, no freezing occurred. For instance, subtracting the heat capacity of 0.875 gm. of dry gelatin from the heat capacity per gram of the 87.5% gel would give the heat capacity of 0.125 gm. of water bound in this gel. It was realized that, if there are different stages or degrees of binding, the heat capacity of one gram of bound water in an 87.5% gel would not necessarily be the same as the heat capacity of one gram of bound water in a more dilute gel. But it was thought that measurements on a gel containing nearly the maximum amount of non-

freezable water should give a value for the average heat capacity of the water which could be applied to the unfrozen portion of the water in more dilute gels.

This was based on the assumption that that water which is bound in such a way as to remain unfrozen would remain similarly bound at the final temperature of the calorimeter, 25° C. Inspection of the 0° isothermal in Fig. 5 reveals a flaw in this assumption. It is to be expected that the specific heat of the bound portion of the water should be less than 1, since bound water presumably should possess fewer degrees of freedom than free water. Hence the average specific heat of the water contained in a gel, which includes bound water, should be less than 1. In other words, the heat capacity per gram of the water in a gel, even above the freezing point, should be less than that of free water. This inference was verified for the 87.5% gel, but in all the others the heat capacity of the water between 0° and 25° C. proved to be greater than that of free water. This is apparent in Fig. 5 and is also shown by Table VI in which are given the average specific heats obtained by dividing the heat capacities in the third column of Table V by 25.

TABLE VI

AVERAGE SPECIFIC HEAT OF WATER IN GELS BETWEEN 0° AND 25° C.

% Gel	9.0	20.9	24.0	45.3	58.1	61.4	67.7	87.5
Av. sp. ht.	1.03	1.07	1.08	1.24	1.19	1.11	1.38	0.80

The heat capacity of a gel between 0° and 25° C. is greater than the sum of the heat capacities of the water and the gelatin. The increase is proportional to the amount of gelatin per gram of water up to a gel concentration of 45%, since the 0° isothermal of Fig. 5 is a straight line up to that point. Putting it in another way, the increase in heat capacity per gram of gelatin is constant up to a gel concentration of 45%, which is apparent from Fig. 4, where the 0° isothermal is also a straight line within this limit of concentration.

Evidently the process of cooling a gel from 25° to 0° C. results in some change in the gelatin-water relation which is accompanied by the evolution of heat. This might result from an increased binding of water as the temperature is lowered. That this is possible is shown by the work of Neville, Theis and K'Burg (10). They have found that the volume contraction of gelatin gels during the process of swelling increases with lowering of the temperature. Such an increased volume contraction apparently means greater hydration or firmer binding. This process should be accompanied by the evolution of heat since the imbibition of water by gelatin is an exothermic process. Upon warming the gel from 0° to 25° C. the converse would take place, namely the absorption of heat. Hence the measured values for the heat capacities at 0° C. would include this effect, which would explain the unexpectedly high values for the apparent specific heat of the water.

The average specific heat of the water in a 67% gel is found to be greater than 1 down to −80° C. This may be due entirely to the increase in intensity of binding continuing throughout this range of temperature or it may be due to a gradual freezing of part of the water. From the shape of the heat capacity curve (Fig. 3) for this gel, it is impossible to state definitely that there is no freezing. Certainly no break in the curve appears. But there is the possibility of a gradual freezing similar to that which appears to take place in the more dilute gels between the temperature where freezing begins and about −25° C. Some evidence that no freezing occurs was obtained by measuring the heat capacity of this gel at −10° C., first by previous immersion in a bath at −78.5° C., and then by cooling slowly to −10° C. from room temperature. In the latter case supercooling rather than freezing should occur. All the results, however, fell on the same smooth curve.

Another anomalous result of the measurements on the 67% gel is that its heat capacity at 0° C. proves to be greater than that of the 61% gel. This may be seen by reference to Fig. 3 and Table II. The following is advanced as a possible explanation. It has already been suggested that a lowering of the temperature from 25° to 0° C. results in an increased intensity of binding, accompanied by the evolution of heat. The amount of heat evolved during this process will depend on the intensity of the binding forces brought into play (12). In a 67% gel at 25° C. all the water is presumably more or less bound by the gelatin, but as it is cooled to 0° C. an increase in the intensity of binding occurs and heat is evolved. If "binding" is pictured as a surface phenomenon occurring at the boundary of the gelatin particles, it may be assumed that not all the available surface forces are being employed by water molecules at 25° C. As the gel is cooled, additional surface forces are brought into play. Now if it is assumed that, when more water is added at 25° C. to form a more dilute gel, these additional surface forces are brought into action at that temperature, it follows that the increase in intensity of binding on cooling the gel to 0° C., and the consequent evolution of heat, will be less than in the case of the more concentrated gel (compare Ref. (2), page 176). This would be in accord with the experimental observations.

The calculation of bound water from calorimetric measurements is thus complicated by the fact that when the gel is cooled, there occurs, accompanied by evolution of heat, some change in the gelatin-water relation which can hardly be identified with the freezing of water in the gel since it is observed above 0° C. If the same heat effect continues below the freezing point, it is experimentally indistinguishable from the latent heat of freezing. The existence of this effect was quite unsuspected at the beginning of the present research and it has not been recognized at all by previous investigators. Robinson (11), for instance, calculates the specific heat of tissues by means of the formula, $S = h_w f_w + h_s f_s$, where h_w and h_s are the specific heats of water and of the solid portion of the material respectively and f_w and f_s the respective fractions of the total weight. It is evident from Fig. 5 and Table VI that this

equation is not applicable to gelatin gels and this fact renders doubtful its validity in the case of other colloidal systems.

It is clear that the measurements on the 67.7% gel do not provide the hoped-for basis for the estimation of the average heat capacity of the bound water. Values based on the results for the 87.5% gel might be used as was done in the tentative calculations of the previous paper but this procedure involves some rather doubtful assumptions. The complete interpretation of the data here presented calls for further measurements in the region of 50–80% gel concentration and also for detailed and accurate measurements of the heat of swelling at different temperatures. The only figures for the heat of swelling at present available are those of Rosenbohm (12) which were obtained with a Bunsen ice calorimeter and apply only to the temperature 0° C. Further work along these lines is contemplated and it is hoped that the required data may be obtained.

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GRAVITATIONAL AND MAGNETOMETRIC SURVEYS OF THE ONAKAWANA LIGNITE AND GRAND RAPIDS SIDERITE DEPOSITS[1]

By A. H. Miller[2]

Abstract

A traverse about three miles in length was made with a torsion balance over the lignite deposit at Onakawana in northern Ontario, where it has been outlined by extensive drilling over an area of a few square miles. The drilling shows that it is overlain with 50 to 100 ft. of boulder clay and is imbedded in Cretaceous clay of considerably greater density than the coal. The traverse crossed the seam in three places. At each crossing a definite lowering in the value of gravity was observed. The boundary of the deposit was shown along two parts of the traverse by the large and definite effect on the gradient of gravity. Owing to the fact that as a rule the stations were as far as 100 metres apart and that the drill holes were usually 1000 ft. or more from one another, uncertainty exists at some stations regarding the respective gravitational effects of variations of the coal seam, on the one hand, and possible irregularities in the boulder clay, on the other. The results indicate that the torsion balance may be of use in outlining such deposits as the Onakawana lignite. A better estimate of the value of the instrument could be made if it had been possible to make a more definite test of the effect of irregularities in the boulder clay.

Numerous magnetometer readings over and in the vicinity of the coal seam showed so little variation that there appears to be little hope that this instrument would be of use in prospecting for the coal, at least in so far as the immediate boundaries are concerned.

A preliminary survey of the Grand Rapids siderite deposit on the Mattagami river was made with vertical and horizontal magnetometers. The iron occurs in limestone which, except at the river, is buried beneath 50 ft. or more of drift. The anomaly produced by the iron does not apparently exceed 100 γ and is complicated by a larger and more regional anomaly which makes definite interpretation of the results difficult. A more detailed survey might be productive of useful results. Magnetometric traverses were made along the rivers from Grand Rapids to James Bay and from Williams Island in the Abitibi to its mouth. A similar magnetic anomaly to that at Grand Rapids was observed at Williams Island. There is quite a marked magnetic anomaly at Moose Factory.

Introduction

This paper contains an account of geophysical investigations by the Dominion Observatory during the season of 1931 at Onakawana, Ontario, and vicinity, in co-operation with the Ontario Research Foundation and the Ontario Department of Mines. Part I deals with the main investigation which consisted of a torsion balance survey of part of the lignite field at Onakawana, having for its object the determination of the applicability of the instrument to the delineation and possible discovery of such deposits.

While the above survey was in progress, magnetometric traverses of the lignite at Onakawana were also made, and a preliminary magnetometric survey was made at Grand Rapids and along the Mattagami, Moose, and Abitibi rivers. The purpose of this latter work was in the first place, if possible, to outline the boundaries of the siderite deposit at the Grand Rapids of the Mattagami river about 25 miles from Onakawana. Failing this, it

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Contribution from the Dominion Observatory, Ottawa, Canada.

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was hoped that sufficient preliminary work would be completed in the time available to make it possible to form an estimate of the applicability of the magnetic method. A report on this work appears in Part II.

The observations with the instruments (except where the contrary is indicated) were taken by the writer. The work in the field was planned and carried out with the joint co-operation of Mr. R. H. Hawkins, Physicist, and Dr. W. S. Dyer, Geologist, representing the above-mentioned institutions. The torsion balance work was done with the Askania instrument of the Geological Survey, and the magnetic work with three Ad. Schmidt variometers of the Observatory and a fourth belonging to the Geological Survey.

The muskeg, standing at an elevation at Onakawana of 175 ft., is a flat surface, having an average fall in elevation northwards of about three feet per mile. At one time following the retreat of the ice sheet, which left a drift deposit over the coal of from 60 to 100 ft., it formed part of James Bay. The submergence below sea-level for a long period resulted in the smoothing out of the top surface of the boulder clay, and left a layer of marine clay about 10 ft. thick. Since the retreat of the sea there has been deposited on the marine clay in recent geologic times the muskeg, which is, as a rule, about four feet in thickness and consists of decayed vegetation and fallen trees. It is partly wooded. The density of the spongy, mossy material forming the upper surface does not exceed 0.2, and except in very dry weather it is water soaked to the top. It presents a springy surface which is pressed down from six inches to a foot at each step, and there is no rigidity offered by the whole four-foot depth of the muskeg.

Owing to the lightness of the lignite compared with the clay in which it is embedded at Onakawana, the possibility is suggested that the torsion balance might be applied with success to the location of deposits of lignite similar to that outlined by drilling at Onakawana. Other conditions favorable to success with the instrument are presented by the very flat terrain, and by the fact that the thickness of the coal seam approaches a magnitude comparable to the depth of overburden, which varies from about 50 to 100 ft. Factors that might operate against success are possible lack of uniformity in the overburden coupled with the possibility of the coal thinning out so gradually from the main body that only comparatively negligible effects might be produced. It was, of course, to determine how far the favorable conditions were offset by the unfavorable influences that the survey was undertaken.

With the exception of the shallow river valleys the geology of the country for many miles around Onakawana is for the most part concealed beneath a mantle of drift. The area thus offers particular scope for prospecting by geophysical methods.

Figs. 1 and 2 show the conditions under which the coal is found, and give an indication of the gravitational effects that may be expected from the lignite. Fig. 1 is taken from a section supplied by Mr. T. F. Sutherland, Acting Deputy Minister of Mines, Toronto. The densities are averages of several determinations in each case by Mr. R. H. Hawkins. Fig. 1 shows

only the geology near the surface, which is, in all likelihood, responsible for the greater part of the local gravitational effects in the vicinity of the coal. Depending on the inclination and continuity of the strata, comparatively less local effect may be expected from the contact (Fig. 2) of the Cretaceous with the Devonian. More regional effects may be produced by the more deep-seated geology but it is probable that these effects, if they exist, are quite small in the region covered.

Fig. 2 is a reproduction of Fig. 3 of Dyer's paper (1) on the stratigraphy and structural geology of Moose River basin. Information on the geology of the deposit may be obtained from Dyer's papers (1, 2).

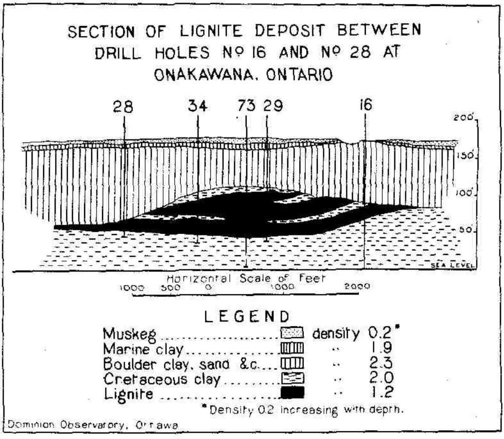


FIG. 1

Part I—Torsion Balance Survey at Onakawana

Setting Up the Instrument

Setting up a delicate instrument for the purpose of observation became the first problem in connection with the work. A satisfactory method was as follows. Three-quarter inch metal rods, about 7 ft. in length, were pushed through to the marine clay and then driven down 18 to 24 in. into it. The tops were set in the form of a triangle and thus formed a tripod with the base plate of the instrument. The tops of the rods were braced with a wooden triangle and a metal wire carrying a turnbuckle. Even on moderately windy days the set-up was slightly disturbed by vibration. A visual instrument was tried but could not be used on account of the vibration caused by the move-

ments of the observer after he had entered the hut. It is possible that with a hut of different construction it might be practicable to use this type of instrument.

Computations of the terrain effect at over 50 stations established the fact that at stations in the open muskeg the effects are so small that they can be safely neglected.

Observations and Discussion of Results

Fig. 3 discloses a number of sharp and rather irregular changes in the observed gravitational effects. This is particularly true of stations in the vicinity of D.H.* 31 and, roughly speaking, of those lying between it and D.H. 19. These results indicate corresponding irregularities in the subterranean densities. Whether these are attributable to variations in the nature of the drift itself, or in the thickness of the coal seam, or a combination of both, is very difficult, if not impossible, to determine from the survey. As we approach D.H. 32 and follow the survey along to the terminus at D.H. 28 there is less irregularity. This is particularly true of the results where the coal seam is thickest, that is, to the north of the Onakawana river in the vicinity of shaft No. 4. Southern boundaries of the coal in apparent agreement with the results of drilling are indicated by the large effects observed in the vicinity of D.H. 26 and 16. With the exception of the gradients observed between D.H. 33 and 29 (these may be caused by an increase in the overburden, a possibility indicated by D.H. 35), the gradients over the area south of the Onakawana are small, and that at the station to the south of D.H. 28 is in agreement with the thickening in the boulder clay and thinning in the coal seam revealed by the drill hole. Generally, the magnitude and direction of the H.D.T. over the area in which the thick seam is found, agree with that to be expected from the information supplied by the drilling.

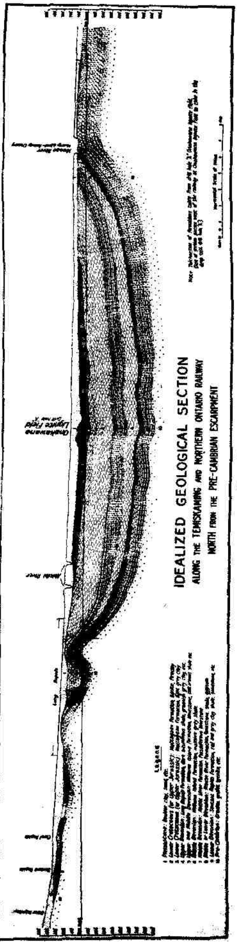


FIG. 2

With one exception, the gradients and H.D.T's between the station due east of shaft No. 1 and D.H. 20 are small and maintain on the average uniform directions. If there is coal in the vicinity of the traverse, the indication is that it lies to the east. In agreement with the drilling a barren area is indicated in the vicinity of D.H. 30. It is probable that the gravitational effects in

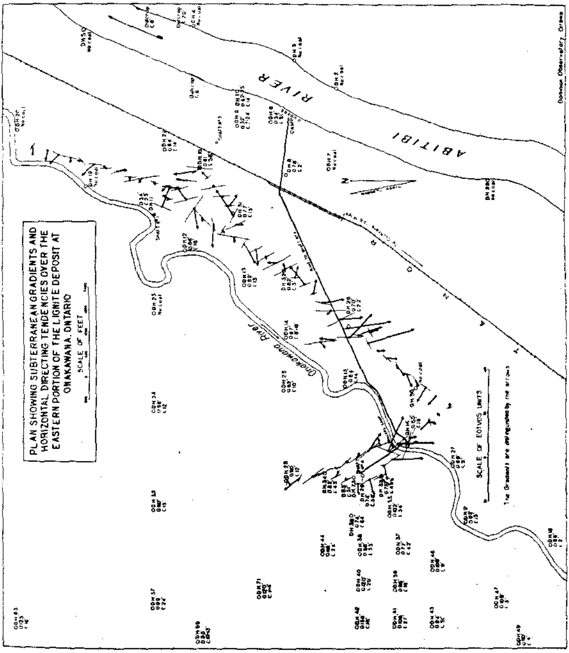


FIG. 3

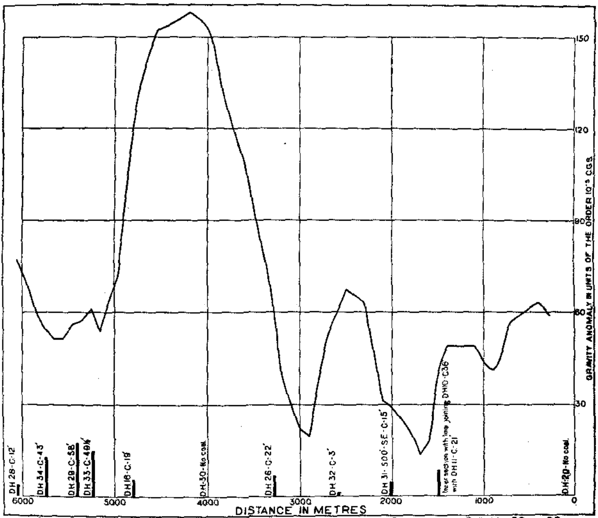
this area are due mainly to irregularities in the boulder clay, and it is likely that the traverse of this region gives a better indication of the nature and magnitude of these particular effects than does any other part of the survey. Along the main traverse in the vicinity of D.H. 31 there are two stations in which there are sudden reversals in the curvature amounting to about 40 Eötvös units. Without the establishment of other stations near them, it is

difficult to say to what they are due. They might be due to local thickening of the seam of about 20 ft. or to the overburden changing from clay to either sand or gravel. If a channel with vertical edges (length great compared with its width) were imagined near the top surface of the overburden, then it would be necessary for the channel to be just so deep that the side of it subtended an angle of about 20° at the stations in order for it to produce the gravitational effect mentioned in the curvature. This could be accounted for in many ways. For example, a channel in the boulder clay filled with sand or gravel of thickness 14 ft. immediately below the marine clay with edges at horizontal distances of 14 ft. on either side of the station would be sufficient to produce the effect. Furthermore, the effect would be practically negligible at external points more than 85 ft. from either edge. A thickening of the coal seam is likely to take place at the expense of the boulder clay, thus bringing the coal seam nearer the surface. Owing to this fact and to the fact also that faces of a seam in the form of a rectangular block produce opposite gravitational effects at points outside the block, a narrow seam of coal will produce quite a local effect too. A local thickening of 15 ft. over a width of 120 ft. in the main coal seam would produce the required effect at a station situated over the centre, and an effect of not more than 10 Eötvös units at external points 60 ft. from either edge, and of course still less than this for points farther distant. D.H. 31 is evidently situated on the easterly edge of a disturbance and so placed that the maximum effect is produced upon the gradient. This may be due to the same cause that results in the large curvature in the second station to the north.

Cross Traverse at Station 35 or D.H. 26 and Stations in their Vicinity. In considering the results for this short traverse and those in the vicinity of D.H. 16, it may be remarked that the magnitudes of the gradients and H.D.T's are such that they might be caused by the amount of overburden and coal indicated at the drill holes, which, at D.H. 26, is 70 and 22 ft. respectively. For an abrupt boundary at D.H. 26 there would theoretically be a maximum gradient of about 44 Eötvös units, which is very close to the observed value of about 50. However, the rate of change of gradient and the H.D.T. are too gradual to be in accord with the conditions specified above. To satisfy all the observed requirements of an abrupt boundary, it would be necessary that the mean depth of the material causing the disturbance should lie at a depth of about 100 metres. For example, a block of coal 31 metres thick with an overburden of 86 metres would be required to account for the effects. The existence of such a block is, of course, not likely. If we suppose that D.H. 26 is on a sloping edge of coal, as it appears to be from the observations, and that in the vicinity to the north of the drill hole the seam thickens, it can be shown that theoretically the observations could be approximately satisfied by a seam with a sloping edge of about 1 in 10, and a thickness of about 50 ft. The effects at the two most northerly stations, owing to their distance, can be only slightly affected in so far as the gradient is concerned by the assumed conditions in the vicinity of D.H. 26. The results at these stations

represent new conditions that are encountered in their vicinity. Qualitatively at least an increase extending for a considerable lateral distance in the mass of the boulder clay before reaching the last station and a decrease at the last station would produce the observed effects.

The Gravity Anomaly Curve. Fig. 4 presents the integrated effects of the gradients along the main traverse, obtained by multiplying the average value of the gradients resolved along the traverse by the distance between the stations; the values represent the differences in gravity between the



Graph showing Relative Values of Gravity along Main Traverse of Onakawana Lignite Deposit between Drill Holes 20 and 28

Fig. 4

stations reduced to a common elevation, and corrected for terrain, topography, and latitude. A curve drawn through the tops of the columns (Fig. 4) representing the thickness of the coal, would form a more or less inverted image of the gravity anomaly curve. The coal regions are regions of low gravity and the barren areas of high gravity. In the region of thickest (most) coal, to the left in the figure, notwithstanding the fact that there is a large relative (compared with the immediate vicinity) fall in gravity, it is not the lowest value when compared with other parts of the traverse between D.H. 26 and 20. Most likely this is due to accumulated error along the traverse.

(particularly in parts where the gravity effects are large) in computing the value of gravity. The closing error of the loop of stations through D.H. 10 and 31 was found to be large, *viz.*, 80×10^{-5} c.g.s. units. The closing of the loop at shaft No. 4 was more satisfactory (less than 20×10^{-5} c.g.s. units). The closing errors of the stations situated on the two loops have in each case been distributed evenly around the loops, and the adopted values have been adjusted to satisfy this requirement. In the loop passing through D.H. 10 the stations are too far apart to give reliable differences in gravity, particularly if instead of being arranged in the form of a closed loop they had been laid out in a linear traverse.

It is interesting to note that the fall of gravity between D.H. 30 and D.H. 29 (shaft No. 4) is just sufficient to account for an extensive seam of coal 60 ft. in thickness.

Conclusion

While sufficient work has not been done to afford a complete solution of the problem, a great deal has been learned. In searching for new deposits and in the detailed outlining of known deposits, the torsion balance may have some application. Indication of favorable areas which, in the final analysis, might or might not contain coal, might reasonably be expected. In exploratory work it would be advisable to place the stations closer together. In a traverse, two lines with the stations at staggered intervals would probably be best. It was found when working with one instrument that 10 to 12 stations could be established in a week. Working with two instruments a party of four could easily run in a week's time a traverse of one kilometre with stations at 50 metre intervals. Before undertaking any extensive exploration it would be desirable to do some further work in a similar drift-covered region where it is certain there is no coal or where the thickness of the coal is well established from numerous drill holes. Although the nature of the topography simplifies the work by eliminating the necessity for terraining, this is offset by the fact that it is difficult to get about in the muskeg, at least in that part of the season best suited for the work. In view of this and the fact that observations with the torsion balance are a slow, tedious process, and keeping in mind the nature of the results obtained so far and that the instrument is as a rule better suited for investigation in detail, it is doubtful whether any extensive exploration with the instrument is to be recommended, except in regions where there is a fair probability of finding coal, and then only in close co-operation with a geologist in order that useless efforts may be avoided and due consideration given to the geology of the region in so far as it is known.

Part II—Magnetometric Surveys

Magnetometric Survey of the Onakawana Lignite Deposit

Traverses of the coal seam with a vertical magnetometer were made by Mr. R. H. Hawkins. Little variation in the intensity was noted. The small anomalies that were observed did not appear to be related to the existence of the lignite.

Grand Rapids Siderite Deposit

The results of the observations at Grand Rapids are shown in Figs. 5, 6, and 7. In Fig. 5 the contact of the ore with the limestone is along the river bank at the point marked *C*, and extends along the bank for a few feet to the east of pit No. 1. It outcrops again between *E* and *F*. The ore forms

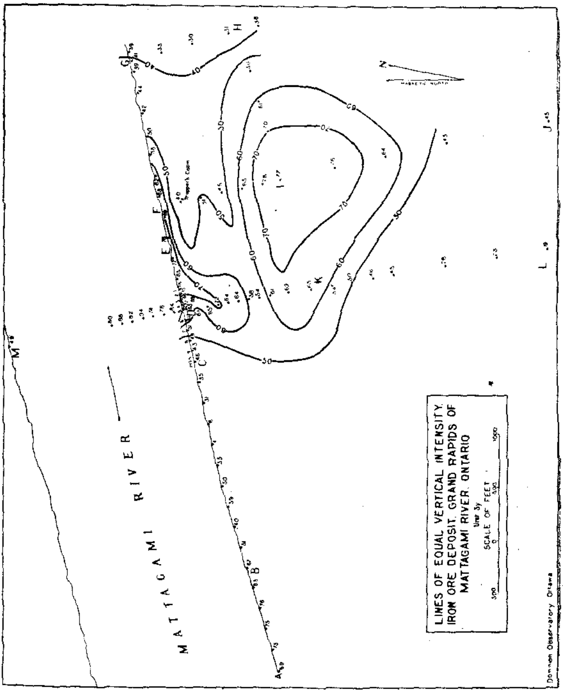


FIG. 5

part of the river bed. The observations in the river were taken on ore in the shallower parts at low water. Immediately to the south of the river bank, the ore disappears under a heavy covering of drift. Between *A* and *B* the bank is formed of ferruginous sands and several iron concretions lying loose along the bank were noted by Dr. Dyer.

The ore is only slightly magnetic and varies in magnetic susceptibility. Samples measured by an approximate method gave values ranging between nearly zero and 350 × 10^{-6} c.g.s. units. Assuming the ore to be uniformly magnetized, the maximum vertical effect over the body would not likely

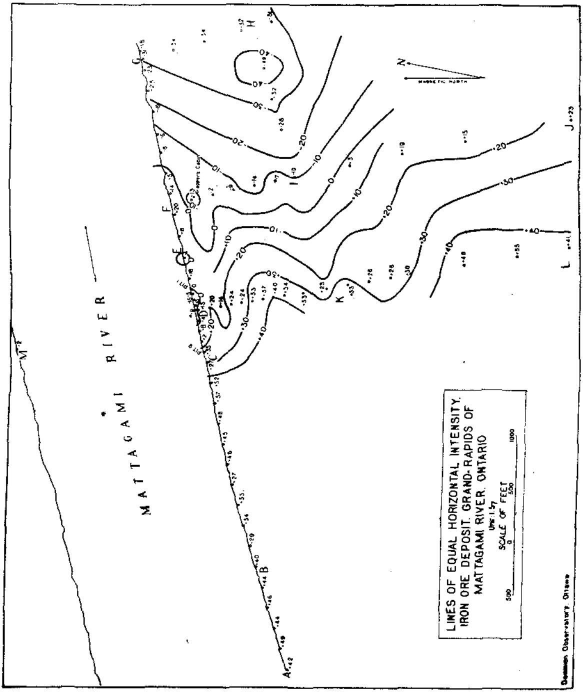
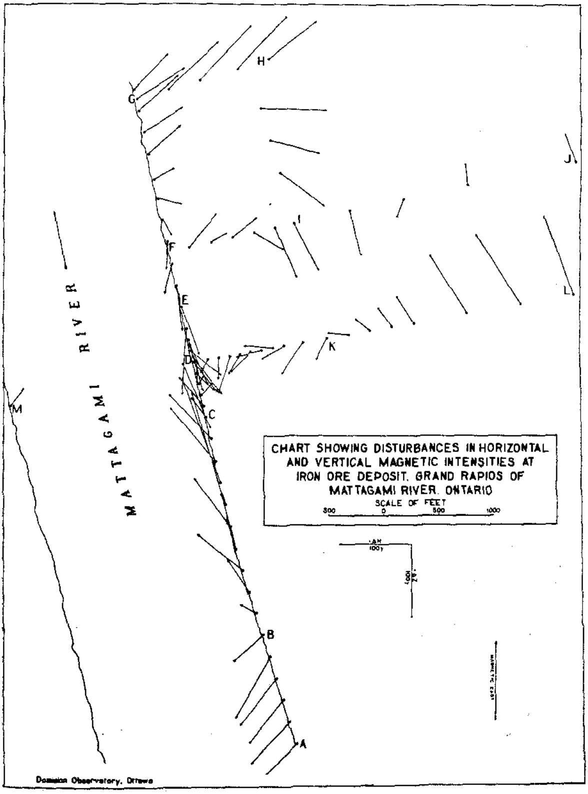


FIG. 6

exceed 140γ, taking the susceptibility to be not greater than the above value and with the point of observation far enough from the ore boundaries to escape effects due to magnetic charges on the sides of the ore body. The observed maximum positive anomaly produced by the ore does not apparently exceed 100γ.

Values given in Figs. 5 and 6 are expressed in terms of one-tenth division of the corrected readings of the respective instruments. For Fig. 7 the

normal values were determined by averaging the values obtained at 500-ft. intervals along the main traverses indicated by the lettering. The adopted normal values are 55 for the vertical intensity and 13 for the horizontal. Fig. 5 discloses three regions of high vertical intensity, one between *A* and *B*, another occupying the greater part of the area covered by the outcrop of ore, and a third centering around *I*. Fig. 7 indicates that the cause of the



disturbance between A and B is located in the direction of the river and that the cause of the disturbance at I is more deep-seated than that of the disturbance south of D. The centre of disturbance at D is indicated at a depth of about 200 ft. which includes up to 100 ft. of drift. It is not likely that the magnetic effect of the iron produces all the anomalies indicated on the charts. The effect of the iron is apparently small and for the most part is masked by a larger and more regional effect. However, it may be that the effect to the south of D is due to the iron, and that the extension of the first 55 contour line immediately to the south of the river indicates approximately the southern boundary of the ore. On the other hand, it is clear that the ore exerts little magnetic effect, perhaps too little to render its detection certain. A more detailed, methodical and careful survey laid down in the form of a grid might lead to more certain results.

Records at 15 stations where observations were repeated from two to nine times, show that the probable error of a single observation with the vertical magnetometer was 0.5 scale division, equivalent to an error of 15γ. The corresponding error with the horizontal magnetometer was 0.6 scale division, equivalent to 9γ. The larger probable error with the vertical instrument may be due to the fact that the base instrument available for the vertical measurements was less sensitive than the corresponding horizontal instrument, the scale values in the two cases being 60 and 15γ, respectively.

The River Traverses

A traverse with stations 500 to 1000 ft. apart was run to a distance of about 2½ miles southerly from J in the direction of $I\ J$ to investigate the regional effect that was apparent in the results already obtained. It was found that with minor fluctuations the horizontal intensity kept increasing and the vertical decreasing (Fig. 8).

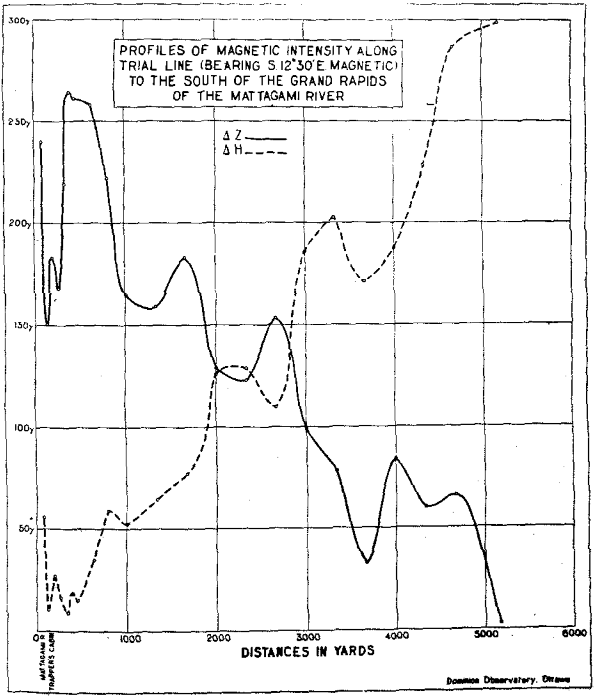
Along the rivers are encountered geological conditions similar to those prevailing at Grand Rapids (particularly at Williams Island on the Abitibi) where there is no reason to suspect the presence of ore. The results, not corrected by base instrument, are shown in Figs. 9 and 10. The readings were corrected from observed variations in intensity observed at Agincourt.* The normal or zero value for the river traverses was obtained by taking from the curve in Fig. 9 the observed values at intervals of 2½ miles. Values were corrected for the latitude variations in the magnetic intensities, as obtained by a least square solution from the values of the observed intensities at New Liskeard, Mattawa, Ft. Albany, Moose Factory, and Cochrane.** In making the solution no account was taken of the longitude variation. The result of the solution gave 9.1γ per mile for H and 6.8γ per mile for Z. The results

**Supplied by the Director of the Meteorological Service.*

***The values for these places were supplied by Mr. C. A. French of the Dominion Observatory staff.*

reveal a decided local anomaly at Moose Factory at about one-half to three-quarters of a mile south along the water's edge from the Hudson Bay post. The ore exposure at Grand Rapids is on an anticline whose axis runs almost due east and west. It is exposed again on crossing the Abitibi River at Williams Island, about 11 miles east. Figs. 9 and 10 show a similar magnetic effect at the two crossings.

The positions of the points of observation along the river were obtained by projection along the astronomical meridian. It must be borne in mind in interpreting the results that the profiles are not the ideal ones along the magnetic meridian. The horizontal anomaly and the resultant disturbance



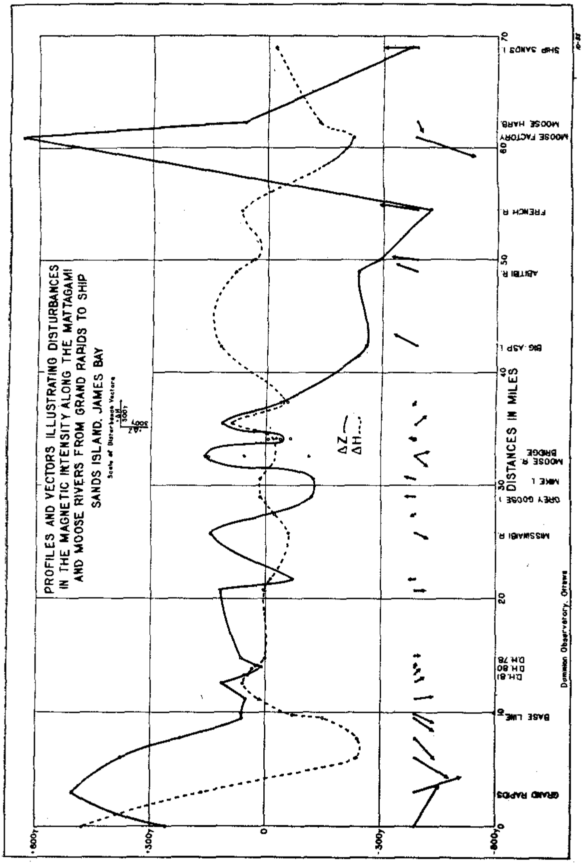

PROFILES AND VECTORS ILLUSTRATING DISTURBANCES IN THE MAGNETIC INTENSITY ALONG THE MATTAGAMI AND MOOSE RIVERS FROM GRAND RAPIDS TO SHIP SANDS ISLAND, JAMES BAY
ΔZ
ΔH
DISTANCES IN MILES
GRAND RAPIDS
BASE LINE
D.H. 78
D.H. 80
D.H. 81
MISSINAIBI R.
GREY GOOSE I.
MIKE I.
MOOSE R. BRIDGE
BIG ASP I.
ABITIBI R.
FRENCH R.
MOOSE FACTORY
MOOSE HARB.
SHIP SANDS I.
+600γ
+300γ
0
-300γ
-600γ
Dominion Observatory, Ottawa

FIG. 9

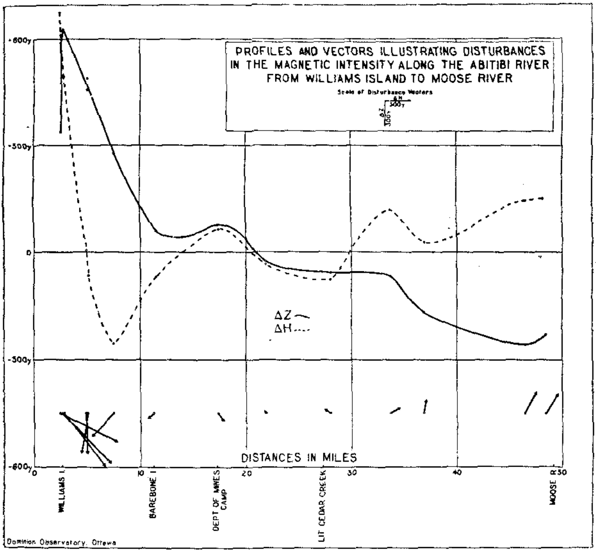


FIG. 10

are in each case disturbances in the plane of the magnetic meridian, and not in the plane of the traverse. This also applies to the iron ore deposit at Grand Rapids (Fig. 7).

CONCLUSION

The results of the magnetic observations at Grand Rapids give some, though indefinite, indication of a southern boundary of the ore a few hundred feet from the river bank. The magnetic anomaly produced by the ore body is small, and is complicated by that due to a more deep-seated cause. If further magnetic work is contemplated, it would be best to plan the observations to cover the ground in the form of a grid, with one of the directions in the magnetic meridian. Accurate determinations of the temperature coefficients and of the scale values of the instruments from time to time would be essential, and it might be found advisable to repeat, preferably on different days, all or most of the observations. The diurnal variation might be evaluated either by an independent observer with another set of instruments at the base, or by returning at frequent intervals to a selected base point with the

field instruments. Even in the former case observations with the field instrument should be taken at the beginning and end of each day at some selected point in order to follow the changes that take place in the instruments themselves. To survey a square mile with observations at 200-ft. intervals, obtaining both magnetic components and possibly also the declinations, would require about 30 days; at 100-ft. intervals, about four months.

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REVIEWS AND NOTES

A CONVENIENT TUBE ELECTROMETER[1]

By V. G. Smith[2]

Recently the School of Hygiene of this university desired to make routine pH measurements using a glass electrode, and it was preferable to make use of the Cambridge potentiometer and Leeds and Northrup 2420–D galvanometer already available. As this will probably be the situation in many cases the circuit used may be of considerable interest.

Convenience in use was kept in mind in designing the circuit. No attempt was made to compensate for battery e.m.f. changes (3, 4) or variations in filament emission (1). It was found that in 15 min. or less the drift of the galvanometer was so slow that it did not interfere with the measurements. Of course this requires that the battery be well charged and then discharged to a condition of steady voltage. The same thing applies to the potentiometer battery.

The circuit is shown in Fig. 1, which also shows the method of connecting to the potentiometer. The tube is a General Electric F.P.–54, supplied entirely from a 12-volt battery. A milliammeter (100 ma.) with a red line at 90 ma. permits the accurate adjustment of the filament current and hence of all voltages.

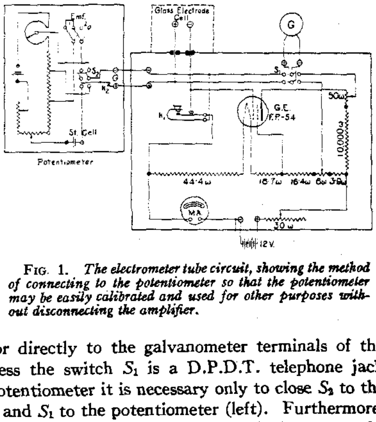

Fig. 1. *The electrometer tube circuit, showing the method of connecting to the potentiometer so that the potentiometer may be easily calibrated and used for other purposes without disconnecting the amplifier.*

By means of the switch S_1 the galvanometer may be connected either in the plate circuit of the tube or directly to the galvanometer terminals of the potentiometer. For neatness the switch S_1 is a D.P.D.T. telephone jack switch. To calibrate the potentiometer it is necessary only to close S_2 to the calibration position (down) and S_1 to the potentiometer (left). Furthermore, with S_1 to the potentiometer (left) and using e.m.f. terminals No. 2, the potentiometer and galvanometer may be used for other work without disconnecting any wires.

[1] *Manuscript received January 24, 1934.*
Contribution from the Department of Electrical Engineering, University of Toronto, Toronto, Ontario, Canada.
[2] *Assistant Professor of Electrical Engineering, University of Toronto.*

When the instrument is used as an amplifier the galvanometer is connected to the plate circuit by the switch S_1 and balanced to zero by means of the two potentiometer rheostats. The 6-ohm rheostat is the coarse adjustment and the 50-ohm rheostat the fine. By short circuiting e.m.f. No. 1 a known potential difference may be obtained across the galvanometer terminals of the potentiometer. With k_2 closed, the pressing of k_1 inserts the potential difference of the potentiometer and the e.m.f. of the glass electrode cell into the control grid circuit in series. When these two are equal and opposite no change in the plate current takes place and hence there is no change in the galvanometer deflection. With a 1000-ohm galvanometer about 90% of the change in the plate current passes through the galvanometer. The potentiometer therefore indicates directly the e.m.f. of the cell when, with k_2 closed, pressing k_1 does not *change* the galvanometer reading. It does not matter whether the galvanometer actually reads zero or not, only the change is important.

It is convenient to lock k_2 closed. If it is of a non-locking type a lead weight may be used. When this is done S_2 should be moved before S_1 when calibrating the potentiometer, for if S_1 is moved first the galvanometer is connected directly across the potentiometer and a violent, time wasting swing results.

It is essential that the grid circuit shall not be opened when inserting the potentiometer and cell, otherwise a violent deflection takes place as the grid proceeds to take on its free-grid potential (2). This means that k_1 must close the new path before opening the old, which results in a temporary short circuit of the potentiometer and glass electrode cell. When finally balanced this is immaterial, but to minimize the possibility of polarization in the preliminary adjustments it is desirable to have the duration of the short circuit as short as possible. For this purpose the "close-before-open" type of switch is used at k_1, permitting extremely short durations of short circuit and yet assuring the proper sequence of events. Such a switch is shown in principle in the diagram.

The whole circuit is shielded in a copper box and for convenience everything is mounted from the lid. It is necessary that the key k_1 shall be a shielded key. In this instrument it is made of brass and fits snugly into a brass sleeve which is grounded to the box. A bakelite insert for appearance and a bakelite tip for insulation are added.

The stationary contact of the key k_1 is amber insulated and both insulators where the cell wires leave the box are made of amber though only the negative wire had to be so insulated. Wires connected to the control grid are kept clear of all other insulation. The box is reasonably air-tight and provision is made for drying the air with phosphorus pentoxide. Two nipples are provided so that air may be circulated if desired.

A convenient method of testing for insulation leaks is to use a standard cell with a 100 megohm grid-leak in series with it in place of the glass electrode cell. As the correct e.m.f. is known any errors caused by leakage may be detected.

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THE EFFECT OF A MAGNETIC FIELD ON THE LINEAR RATE OF CRYSTALLIZATION[1]

BY E. W. R. STEACIE[2] AND C. F. B. STEVENS[3]

Abstract

An investigation has been made of the effect of a magnetic field of about 5000 gauss on the linear rate of crystallization from supersaturated solutions of sodium thiosulphate and nickel sulphate. The field had no appreciable effect in the case of sodium thiosulphate. Nickel sulphate showed a slightly increased rate of crystallization in the field.

Introduction

It would be anticipated that the rate of crystallization from a supersaturated solution would be affected by any factor which causes a change in the orientation of the molecules (or ions) of solute as they approach the interface. It is therefore of interest to investigate the effect of a magnetic field on the crystallization velocity, since no direct investigation has been made previously.

Kondoguri (1, 2) observed an increase in the rate of nucleus formation in the spontaneous crystallization of certain organic substances in a magnetic field. Roasio (3) found that in a field the growth of certain faces of several inorganic salts was favored, resulting in elongated crystals.

Apparatus

The main portion of the apparatus was a horizontal 5 ft. length of ⅛ in. soft glass tubing, surrounded by a water jacket which could be maintained at constant temperature (20° C.). A hot saturated solution of the substance under investigation was sucked into the long tube and allowed to cool to the temperature of the water jacket. Crystallization was then induced by seeding at the open end.

[1] *Manuscript received April 3, 1934.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Honors Undergraduate, McGill University.*

At one point the long tube passed between the poles of an electromagnet having pole pieces about 4 in. in diameter. The field strength was about 5000 gauss.

Rates of crystallization were determined by observing the time for the crystal boundary to advance through successive 5 cm. intervals.

The main difficulty encountered was the tendency for crystallization to start spontaneously at a number of places along the tube. It was found that this difficulty was very greatly accentuated if the tube were cleaned between runs with any powerful cleaning agent. On this account, therefore, only hot water was used. It was also essential that the solutions used should be free from dust, etc. They were therefore filtered through hot sintered Jena glass filters.

The rate of crystallization varied somewhat from tube to tube. In any run, however, the results were very consistent, and hence the effect of the field could be inferred accurately.

Experimental Results

I. *Sodium Thiosulphate*

The results of five typical runs are shown in Table I. In each case sodium thiosulphate crystals were dissolved in their own water of crystallization at 70° C., and then cooled to 20° C. in the tube.

TABLE I

SODIUM THIOSULPHATE

Interval in cm.	Time in seconds					Interval in cm.	Time in seconds				
	I	II	III	IV	V		I	II	III	IV	V
0–5	150	116	190	209	149	55–60	157	133	181	190	155
5–10	145	119	175	205	139	60–65	159	141	185	223	159
10–15	149	130	188	199	133	65–70	153	158	182	196	135 ↑ Field
15–20	142	123	176	207	151	70–75	150	125	181	212	141
20–25	147	123	184	205	144	75–80	152	126	181	210	143
25–30	148	126	195	204	128	80–85	150	125	180	213	143
30–35	156	137	180	205	137	85–90	165	128	170	217	155 ↓
35–40	153	133	180	202	142	90–95	165	149	183	217	174
40–45	148	127	180	204	140	95–100	170	163	188		145
45–50	155	120	174	201	145	100–105	163	150	185		
50–55	155	135	180	200	159	105–110	146	130	184		
						110–115	145	123	180		

II. Attempts were also made to use supersaturated solutions of ferrous ammonium sulphate, ferric ammonium sulphate, ferric chloride, ferrous sulphate and nickel ammonium sulphate. In every case hydrolysis occurred to some extent, even if acid were added to check it. This resulted in a colloidal suspension which furnished an enormous number of nuclei and caused irregular crystallization all along the tube.

III. *Nickel Sulphate*

Experiments were also made with solutions of nickel sulphate. Great difficulty was encountered in preventing crystallization all along the tube, but a few successful runs were made. These showed widely varying rates of crystallization, but in any individual run the rate was reasonably constant. The data are summarized in Table II.

TABLE II

NICKEL SULPHATE

Saturated at	Cooled to	Supercooling, °C.	Average rate, cm./min.	Average rate in field, cm./min.
29° C.	6° C.	23	0.436	0.472
80	6	74	0.036	0.045
50	8	42	0.083	—
82	6	76	0.057	0.065
82	3	79	0.021	—

It is evident that there is a slight acceleration in the field. The effect, however, is not much larger than the experimental error.

Discussion

The results indicate that there is very little alteration in the linear rate of crystallization in a magnetic field. It might have been anticipated that owing to the field certain orientations of a molecule or ion approaching a crystal would be favored. No such effect is noticeable. This fact may be considered to be additional evidence for the hypothesis that crystallization takes place by a preliminary adsorption followed by the incorporation of the adsorbed molecule or ion into the crystal lattice.

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A MATHEMATICAL TREATMENT OF A THEORY OF RUBBER STRUCTURE[1]

By T. R. Griffith[2]

Abstract

The theory that the elasticity of rubber is due to the heat vibrations of very long chain molecules, bound to one another at occasional points along their length, but able to move freely relatively to one another at all other points, is susceptible of mathematical treatment. In the present treatment it is assumed that the rubber molecule has a restricted rotation about the axis formed by joining two adjacent junction points.

A stress-strain curve has been developed mathematically on this assumption, and this curve, which is a reasonably close approximation to the curve obtained experimentally, serves as a standard with which to compare the rubber stress-strain curve and as a starting point for further mathematical work on the structure of rubber. The discrepancy between the mathematical and the experimental curve is explained on the very probable assumption that there is a wave motion or other vibration along the length of the rotating chain, as well as a rotation of the chain as a whole.

An explanation of the peculiar S-shape of the beginning of the experimental stress-strain curve develops automatically from the mathematically deduced relation between stress and strain, and it is also shown why the S-shape appears to vanish when the calculation of the stress is based on the actual cross section of the stretched rubber.

In addition, the following values, calculated from the above assumption and X-ray data, of certain constants, were obtained:

(i) The average distance between junction points.

(ii) The average length of molecular chain between junction points and, incidentally, the ratio between the number of freely swinging carbon atoms and those bound at junction points. This gives the number of freely swinging carbon atoms on the molecular chain between junction points and an idea of the length of the rubber molecule.

(iii) The quantity of kinetic energy per cubic centimetre causing the elastic effect in rubber.

(iv) The percentage of sulphur necessary to form the junction points in vulcanized rubber and, consequently, the minimum quantity of sulphur needed for vulcanization, both for hard and soft rubber. This minimum agrees closely with practical experiment.

A brief consideration of the work that has been done on the structure of rubber convinces one that the elasticity is wholly, or at least mainly, explained by a consideration of the kinetics involved.

The fact that when a strip of stretched rubber, one end of which is free, contracts when it is warmed, contrary to the behavior of most bodies, and that it becomes warmed on stretching, commonly known as the Gough-Joule effect (15, 19, 40, pp. 453–461), would lead one to suspect that there is a connection between the kinetic energy of the rubber molecule and its elasticity.

Lundal (25), Bouasse (7), Hyde (18), Somerville and Cope (34), Partenheimer (28) and Whitby and Katz (41) have reported observations, principally stress-strain curves, which show that vulcanized rubber has a lower modulus of elasticity at higher temperatures; *i.e.*, it becomes easier to stretch as the temperature is raised. On the other hand, Schmulewitsch (31), Stevens (37) and Williams (42) found that the elastic modulus increases with the temperature.

[1] *Manuscript received March 14, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.
[2] *Research Assistant, National Research Laboratories, Ottawa, Canada.*

Williams (42) shows that the softening of vulcanized rubber with rise of temperature is due to an increase of plasticity. In order to get rid of plastic flow, he first stretches the specimen several times to within about 50% of its breaking elongation, and then obtains an autographic stress-strain curve of the rubber stretched very quickly. He finds that in this case the rubber actually becomes stiffer with rise of temperature, increasing temperatures causing the stress-strain curves to lean progressively more and more towards the stress axis. He concludes that rise of temperature has two effects, one, a softening due to increase of plasticity, rendering plastic flow more easy, the other, an actual stiffening of the rubber due to rise of temperature. It is not easy to explain the latter effect on any theory which does not take kinetics into account.

It is difficult, for example, to explain the stiffening of rubber with temperature rise by means of the well known two-phase theory, which assumes both a liquid and a solid phase in rubber. In this connection Whitby (39) has shown that no liquid phase has ever been separated from rubber and that the so-called liquid phase which was supposed to be soluble in certain solvents shows the normal behavior of rubber. This phase is elastic, gives an X-ray pattern and will also vulcanize.

It has been shown first by Whitby (39) and later by Staudinger and Bondy (36) that the molecule of crude rubber exists in several different states of polymerization.

The needle molecule theory of Lindmayer (24), which assumes a needle-shaped molecule about 14 times as along as its diameter, as well as the theory which supposes the molecule to be in the form of a spiral (3, 12, 13, 21, 22), postulates a peculiar shape of the rubber molecule itself as the cause of the elasticity and other properties of rubber. In this connection Whitby (39) states that elasticity is shown not only by polymerized isoprene but by polymers of such widely different substances as methyl acrylate, styrene, vinyl acetate, sulphurized fatty oils, and by proteins, and that it can be caused to display itself in materials which are otherwise inelastic by the introduction of swelling agents or the application of heat. It is thus difficult to conceive how the elasticity of rubber can be due to a peculiar shape of the molecule. This shape would have to be the same in all these polymers if either of these theories were correct.

Shacklock (32) developed mathematically, from an assumed structure for rubber, a stress-strain curve which explains the peculiar S-shape of the stress-strain curve for rubber in a manner entirely different from that of the present article, but the development rests upon the assumption of an elastic fibril. The theory developed in the present paper shows how the elasticity of rubber evolves purely from the kinetic motions of the molecules, their inherent stiffness and elasticity being neglected.

The theory of rubber structure mathematically developed in this paper is essentially that of Boggs and Blake (4), who describe rubber as a network of extremely long chains, each link in the chain being a C_5H_8 unit.

Although an exact knowledge of the actual manner of combination of th C_5H_8 units to form a straight chain formula is not essential to the mathema tical development, Whitby (38) showed that there are several scheme possible and, in addition, gives strong experimental chemical evidence in favo of the straight chain, primary valence structural formula for rubber.

In vulcanized rubber, according to Boggs and Blake (4), these chains are bound to one another at points along their length by sulphur atoms. These investigators further assume that the parts of the molecular chains between junction points are in motion relatively to one another and that one of these motions is rotational. They also state that Hock (17) had previously explained the Joule effect on the basis of the orientation of the molecules. Hock, however, though the first investigator to employ kinetics and the orientation of molecules to explain certain phenomena shown by rubber, does not mention rotation of the molecular chain, so that the portion of the hypothesis dealing with rotation is original with Boggs and Blake.

Starting, then, with this hypothesis, the present mathematical development assumes that this rotation is essentially a rotation of the molecular chain as a whole about the axis joining adjacent junction points, that the centrifugal force will cause a tension in the swinging molecular chain, and that a calculable component of this tension will draw the adjacent junction points together. It is assumed that each junction point in the network structure of rubber is joined to all or most of the junction points in its immediate neighborhood through the network, and that consequently each junction point will be drawn simultaneously towards all other junction points around it. The structure of rubber can thus be pictured by imagining it to consist essentially of junction points, connected to one another through a network, and all tending to get closer together, each being prevented from getting too close to adjacent junction points by heat vibrations and by its attraction towards other junction points. An equilibrium is thus established, and the junction points then occupy positions in which the resultant force on them in all directions is zero. If this equilibrium position is disturbed the junction points will immediately, on release of the external stress, return to their former orientation in space, and the body will recover its former shape.

The centrifugal motion, however, is necessarily a restricted one, because there is not enough room for a chain to make a complete turn about its axis without colliding with neighboring chains. Though the rotation is restricted, the centrifugal forces in the rotating part of the chain will nevertheless persist, the chain working its way into the interstices of the structure, away from the axis of rotation, just as surely as if complete rotation were possible.

Boggs and Blake (4), in explaining the Joule effect on their hypothesis, state that "when rubber is stretched, the mechanically applied tension causes at least a partial orientation of the molecules, as is evidenced by the development of double refraction and an X-ray diffraction pattern.

"This orientation due to stretching results in a loss of kinetic energy in the individual molecules on account of the restriction of thermal motion, which instantly appears in the mass of rubber as heat. The other phases of the Joule effect are similarly explained. The retraction of stretched rubber allows the molecules again to assume their random thermal motion, and the energy absorbed in this is drawn from the surroundings by a cooling of the rubber. The application of heat to stretched rubber increases the tendency for molecular motion, which reduces orientation and shortens the rubber accordingly or increases the tension required to maintain the stretched condition."

An additional idea which might be introduced into this conception is that a portion of the energy of stretching does not appear as heat, but is changed into potential energy resulting from the new shape of the molecule. It will be shown later that the tension in a chain rotating about an axis will be increased when the junction points are moved farther apart, as in stretching. This increase in tension, caused by the change in the curvature of the rotating chain, involves an absorption of potential energy which will be given out as mechanical work when the specimen contracts under load, no transformation to thermal energy taking place in the process.

If the rubber were to contract under no load, *i.e.*, snap back to equilibrium, it is evident that this potential energy would be converted into heat energy and tend to heat the specimen. In contraction under no load, therefore, there would appear to be two opposing effects in rubber; a cooling effect due to absorption of heat in the return to random thermal motion, and a heating effect due to the release of potential energy as heat. A quantitative calorimetric measurement of the temperature change of rubber in retraction under no load would appear to be necessary to determine which effect is the greater. In this connection, Gerke (14) has made some interesting applications of the laws of thermodynamics to the Joule effect.

The addition of still another idea to the conception of Boggs and Blake (4) will complete the picture. Heating of the stretched specimen will cause an increase in the kinetic energy of rotation of the chain molecule about its axis, which is the line joining two adjacent junction points. This, as will be shown later mathematically, will cause an increase in the tension in the chain and then cause the junction points to come together along the line of the stress, *i.e.*, will cause the specimen to retract somewhat under constant load. Thus there are two effects of increase in temperature in stretched rubber: first, a tendency to retraction, as conceived by Boggs and Blake, due to the effect of heat in producing the irregularity of the unstretched state, where the molecular arc of rotation becomes bent and twisted into all sorts of shapes and forms; second, a tendency to retraction brought about by increased kinetic energy of rotation which causes increased chain tension. The latter is, apparently, much the greater of the two effects.

The slight cooling noticeable in rubber during the very first stages of stretching is probably the same effect as that observed in all bodies subjected to tension. In rubber, however, the Joule effect rapidly sets in and is sufficient to mask this cooling, so that a heating effect is observed.

Meyer, von Susich and Valkó (27) have recently brought forward a theory of elasticity bearing a similarity to the one just described. They assume that in elongated substances that have a tendency to contract, there are present extremely long primary valence chains which are oriented in the direction of stretching. An essential part of the heat content of these chains consists *of movements of rotation and deformation of individual atomic groups perpendicular to the molecular axis, while the valence vibrations in the direction of the molecular axis have a relatively small heat content. These transverse movements lead to irregular impacts of individual chains perpendicular to the direction of elongation. This results in a pressure of repulsion at right angles to this direction, which in turn causes the tractive tension. The temperature coefficient of tension is shown to be in accord* with this kinetic concept. It will be observed that their theory presumably postulates short molecular arcs and that in the mathematical development of the present theory, the idea of rotation of extremely long molecular chains about an axis formed by joining two adjacent junction points is stressed as the main cause of the elastic effect. It might also be mentioned that the length of, and the number of atoms in, the rotating part of the chain have been calculated mathematically in the present theory.

The enormous swelling of rubber immersed in solvents is explained by the present theory on the assumption that the swelling medium simply interposes itself between the junction points and pushes them farther apart, and (in vulcanized rubber) without disrupting the structure or disturbing the relative orientation of the junction points to each other until after prolonged immersion (5, 39, p. 45).

It should be borne in mind that the theory herein described assumes absolute freedom of movement between adjacent molecular chains at all points except where they are bound together. This means that rubber acts like a liquid throughout most of its structure, and the only part where it may be considered to be solid, or rigid, is at the junction points, where there is no relative motion between adjacent molecular chains. The junction points also move or vibrate relatively to one another. Thus rubber is only a pseudo-solid, its solidity or tendency to hold a definite shape being due only to the equilibrium position of the junction points. Soft rubber, therefore, in some of its properties, acts like a liquid of very low viscosity. This can be inferred from the rapidity with which rubber will snap back to its equilibrium position after being deformed. If the liquid part of the rubber were viscous it would return very slowly. An example of what high viscosity in a rubber structure will do may be found in synthetic dimethylbutadiene rubber (41), which is very sluggish in retraction after deformation, but which when heated to around 70°C. has plenty of snap. There is thus in the present theory a certain similarity to the two-phase theory, but in this case only one size of molecule is concerned in both the liquid and solid phases.

Carothers, Williams, Collins and Kirby (10), in an article on poly-chlorobutadiene, the new synthetic rubber, state that chloroprene polymerizes in two different ways. In one type of polymerization the chloroprene molecules

join on to one another at the ends and form a long chain. Elasticity does not develop, however, until the second type of polymerization, a cross linking of the chains, takes place.

Busse (9), in describing the structure of rubber, mentions cross linking by means of sulphur bonds in vulcanized rubber and by means of intertangling of molecules in crude rubber. He also states that the molecular chains are in thermal motion, without stressing any particular type of motion. He explains how this view agrees completely with the known behavior of rubber.

It would not appear, however, that intertangling alone could account for the cross linking effect in crude rubber. One must also assume the action of van der Waals' forces holding the molecular chains together at the points where, owing to intertangling, they are constantly in contact. A certain amount of intertangling should also exist in vulcanized rubber.

Shacklock (32) describes an experiment with oleyl chloride, sulphur chloride and glycerol which points fairly definitely to the existence of a network structure in elastic vulcanized oils*.

In any event it is apparent that many investigators of rubber structure believe the property of elasticity to develop as a result of cross linking of long molecular chains. The present theory merely adds to this conception the idea that elasticity is the direct effect of the thermal and, in particular, the rotational vibrations of such a network.

As already intimated, it is possible to work out the mathematical consequences of the present theory. What is original about the present mathematical method is that the stress-strain curve is developed from an assumed rubber structure, whereas all previous mathematical treatments of the stress-strain curve of which the writer is aware, with the exception of that of Shacklock (32), attempt to find an empirical formula to fit the experimental stress-strain curve (2, 16, 23). None of these attempts have thrown much light on the structure of rubber because it has been impossible to interpret the true meaning of the equations obtained. The present mathematical development, while not resulting in a formula which fits the stress-strain curve with the accuracy of empirically obtained equations, has nevertheless necessitated the working out of some very interesting constants, and the work has thus thrown considerable light on the structure of rubber.

Explanation of Mathematical Method and Discussion of Results Obtained

General

When a collision between two adjacent chains takes place, rotation will obviously stop for an instant and the tension due to rotation will be momentarily lessened. Immediately after the collision, however, the rotation will increase again and the tension will be resumed. The mathematics neglects this momentary cessation of rotation and η, the kinetic energy of rotation in the molecular chain, is that amount of energy which will account for the elastic effect, assuming the rotation to be continuous. η is, as it were, an average or, rather, equivalent value. Any error in the above assumption, however, will not affect the other constants.

*[illegible]

Though wave or other motion along the chain will also be considere rotation has been taken as the basis of the calculation, because it was thougl that only a structure in which rotation was the prii cipal feature could account for the high elongatioi observed in rubber. For example, Fig. 1 illustrat a wave motion along the molecular chain, the ar plitude of which is advisedly shown as equivalei to that of a semicircle. Such an amplitude is we above the maximum amplitude possible in a wave like structure, especially since the arc ADB, as will be shown later, contain as many as 216 freely swinging chain carbon atoms, and Fig. 7 shows how closely the atoms in rubber are packed.

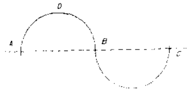

FIG. 1. *Semicircular molecular arc.*

The total possible elongation for the shape of arc given in Fig. 1 could not exceed 57.08%, as the following calculation will show:

$$\text{Elongation, } \% = \frac{100\,(\text{arc } ADB - AB)}{AB} = \frac{100\left(\frac{\pi AB}{2} - AB\right)}{AB} = 57.08\%,$$

assuming the arc ADB to be roughly a semicircle.

On the other hand, in Fig. 2, the arc FHG, rotating in a restricted manner about the axis FG, where F and G are junction points, could have any desired elongation, and shows closer packing; in addition, there is the possibility, as shown in the figure, of a wave motion along the rotating arc. For the sake of simplicity the first attack on the problem will ignore the wave motion, which will be used later to explain the small discrepancy between the calculated curve and that determined experimentally. Further work on this problem will have as its object the incorporation of rotation, wave motion and any other necessary corrections in the one equation relating stress and strain.

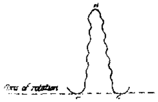

FIG. 2. *Postulated shape of molecular arc.*

Curve B, Fig. 8, was obtained by calculation, Curve A, with a specimen of rubber. Although the curves do not coincide, Curve B serves as a standard of comparison and as a first approximation. The preliminary work has, as already intimated, suggested a means of obtaining a closer approximation by incorporating wave motion. Doubtless still closer approximations will be attempted and obtained in the future until the rubber stress-strain curve is finally brought to an accurate mathematical basis.

Fig. 3 is a mathematical representation of a single rotating chain. The equation for this curve is worked out following the method of Appell and Lacour (1, p. 207-211) in their work on elliptic functions. OA_1O_1 is the molecular chain. O and O_1 are junction points with other molecular chains. The arc OA_1O_1 rotates about the axis OO_1. The distance OB_1 is ξ, the half-distance between junction points. The length of the arc OMA_1 is λ. Values for ξ and λ were obtained by equations worked out by the method of elliptic functions.

As, in order to relate stress and strain, the tension in the chain and the dimensions of the arc must be brought into the same equation, it was neces-

sary, using the general method of Appell and Lacour, to work out an equation for η, the total energy of rotation in the arc, this equation containing both these quantities. The following is the expression for η:

$$\eta = 4A\xi C, \tag{10}$$

where η is the energy in the length of arc, 2λ; A is the horizontal component of the tension in the arc, A being constant throughout the length of the arc; ξ is the distance OB_1, Fig. 3; and C, a variable dependent upon ξ and evaluated by tables of elliptic functions.

The equation for η expresses the value for η in terms of the dimensions of the arc and the tension in the chain, so that the problem then becomes one of expressing the arc dimensions in terms of the actual elongation of the rubber specimen, and the tension in the chain in terms of the stress in kilograms per square centimetre. To do this it is necessary to find, among other things ξ_o, the half-distance between junction points when the rubber is unstressed.

The mathematical artifice used to evaluate ξ_o is to suppose the rubber to be swelled to its maximum extent by, say, a solvent, so that all the molecular chains in the rubber are absolutely straight, and aligned along three axes at right angles to one another, each chain extending from one side of the cube to the opposite side. Every point at which it is possible for one chain to cross another in this system is then taken as a junction point. With the molecules thus regularly arranged it is an easy arithmetical calculation, knowing the total number of chain carbon atoms, and the distance between them from X-ray data (Fig. 7), to calculate the total number of chains, and, from that, the total number of junction points. The rubber is then assumed to contract to its original volume (1 cc.), when the straight chains form arcs owing to heat vibrations and draw the junction points to their normal distance apart in unstretched rubber, thus giving a figure for ξ_o. The degree of swelling permitted in this calculation is regulated by the maximum possible elongation, *i.e.*, the elongation at infinite stress or, rather, the elongation shown by the asymptote of the experimental stress-strain curve—in this particular case the asymptote, LM, of Curve A, Fig. 8. This maximum elongation was 850% and, therefore, the specimen was supposed to have to swell to an elongation of 850% in the directions of the three axes before the chains would be perfectly straight.

The fact that rubber does not swell to such an extent without disintegrating does not disprove the mathematical method. High internal stresses are involved in the swelling of rubber by solvents, and it is not surprising that rupture and disintegration should occur at an early stage of the swelling.

In the structure described above, stretching the rubber in only one direction does not straighten out all the arcs in that direction, those whose axes of rotation are at right angles to the direction of the stress not being appreciably extended. However, the chains on unextended arcs will also align themselves more or less in the direction of the stress, as do fillers which have definite crystallographic axes, thus explaining the X-ray results. Moreover, the

alignment of chains permits the van der Waals' forces to act, thus producin in the rubber a tendency to preserve this alignment, and reducing the tendenc of the rubber to retract. This explains the phenomenon of hysteresis i vulcanized and crude rubber and also the phenomenon of racking.

Now the distance between junction points actually obtained by the mathe matical artifice described above is that in an ideal soft vulcanized rubber stretching to 850% along the three axes. In other words, a theoretical soft vulcanized rubber is being used as a basis of comparison.

The following general expression for ξ_o was found:

$$3.42 \times 10^{-7} \sqrt{\frac{x + 100}{src}} \text{ cm.,} \tag{16}$$

where x is the elongation at infinite stress, in this case 850%; s, the density of the rubber compound, 0.955; r, the percentage of crude in the mix, 93.11%; and c, the percentage of pure caoutchouc in the crude, 95.00% (11). The product, $src \times 10^{-4}$, is the weight in grams of pure caoutchouc in 1 cc. of compound.

The experimental mix used in obtaining the constants, x, s and r, indicated above, is shown in Table I.

TABLE I

RUBBER MIX USED FOR OBTAINING EXPERIMENTAL STRESS-STRAIN CURVE

—	Smoked sheets	Mercapto-benzo-thiazole	Sulphur	Zinc oxide	Stearic acid	Aldol-α-naphthy-lamine	—
Parts by weight	200	0.8	6	5	2	1	214.8
% of total	93.11	0.37	2.79	2.33	0.93	0.47	100.00

Minimum Quantity of Sulphur Required for Vulcanization—Soft Rubber

The total number of junction points in 1 cc. of rubber compound was found to be:

$$2.63 \times 10^{18} \left(\frac{src}{x + 100}\right)^{\frac{3}{2}} \cdot \tag{17}$$

Giving the constants the values shown above, this becomes 6.98×10^{19}.

From this figure the quantity of sulphur necessary for vulcanization may be calculated. Assuming one atom per junction point, the vulcanization of the rubber to the ideal state indicated above would require 0.42% of sulphur in the rubber.

Boggs and Blake (4) state that about 0.5% combined sulphur is necessary and sufficient to produce soft vulcanized rubber. The close agreement between their figure and 0.42%, obtained on theoretical grounds, is more than a mere coincidence. The assumption of two atoms per junction point would make the calculated result 0.84%, which is too high, so that one sulphur atom per junction point is strongly indicated.

The combined sulphur content of the mix under observation, with which Curve *A*, Fig. 8, was obtained, was 2.70% and much higher than the above theoretical result, but a relatively high vulcanization temperature (141° C.) was used. Boggs and Blake (4) vulcanized in the cold, using an ultra accelerator. The much larger quantities of sulphur required in hot vulcanization can be explained by assuming that during hot vulcanization "two main factors are operative, namely: (i) a process of integration, pectization or 'firming up', which consists in (or goes hand in hand with) the chemical process of combination with sulphur, and (ii) a disintegrating or degradation process due to the heat as such." This is the conception of Schidrowitz and Goldsbrough (30). The conclusion to be drawn from such a conception is that if factor (ii) is absent, as in cold vulcanization, factor (i) does not have to proceed as far for complete vulcanization and less combined sulphur will be required.

Quite recently compounds for hot vulcanization have been developed, requiring less than 1% of vulcanizing agent, and it would appear that these compounds, which approach the mathematical ideal with respect to sulphur content, have improved physical properties. This has been shown by Booth (6) and by Somerville and Russell (35).

This explains why, in hot vulcanization, 2–5% of sulphur is needed, while in cold vulcanization about 0.5% may be sufficient. By the depolymerization, degradation, or increase in plasticity mentioned above, one understands on the present theory the breaking of molecular chains. Each break in the chain is mended by the formation of a sulphur bond at, or close to, the broken ends and each rupture requires extra sulphur. These bonds are, strictly speaking, not junction points, for they have freedom of movement, existing as they do on a rotating part of the chain. They might conceivably decrease plasticity somewhat. The calculated sulphur content is low because sulphur combined in this way is not taken into consideration in the calculation.

Minimum Quantity of Sulphur Required for Vulcanization—Hard Rubber

Another interesting calculation is the determination of the quantity of sulphur necessary to make rubber inextensible and inelastic. This is done by making x equal to zero in the expression for the total number of junction points, as follows:

$$2.63 \times 10^{18}\left(\frac{src}{0 + 100}\right)^{\frac{3}{2}} \qquad (17)$$

When x is zero, according to the mathematical conception, the chains are stretched taut between junction points in the unstretched rubber.

Supplying one sulphur atom per junction point gives the value 11.6% for an inelastic compound containing rubber and sulphur only, which is considerably less than the possible maximum of combined sulphur, namely, 32%.

As the chains when in this state of relative immobility have, at room temperature, properties similar to those possessed by the molecules of a substance in the solid state, and as the junction points also form rigid connections between the chains at room temperature, the whole structure should

be solid at room temperature. However, at higher temperatures, owing t plastic flow in the junction points, such a structure may become softened The question of plasticity will be further discussed later.

An experimental verification of this calculation would be difficult. To cause high percentages of sulphur to combine with the rubber, hot vulcanization is required, and while the 11.6% of sulphur was entering into combination at junction points, additional sulphur would be combining at the ends of broken chains to take care of depolymerization, so that the percentage of combined sulphur should be higher than 11.6% for an experimental compound at the point where the rubber just becomes solid and inelastic. It has just been shown that the calculation for soft rubber indicates 0.42% of sulphur at junction points when the corresponding experimental hot vulcanized sample had 2.70% of combined sulphur, or an excess of 2.20% to take care of depolymerization. The excess of 2.20% of sulphur (excluding that portion of the sulphur which combines with the other ingredients of the compound) serves in this case to join up the ends of molecular chains which have become severed through depolymerization. It is not unreasonable, therefore, to assume that when the sulphur at junction points reaches 11.6%, the total combined sulphur would have reached 17%, the point at which rubber is hard at room temperature (20).

The explanation of the apparent lowness of the mathematical result for combined sulphur in inelastic rubber is, therefore, quite simple, and the result does not cast doubt upon the accuracy of the mathematical method. The object having been to calculate the minimum amount of sulphur necessary to produce inelastic rubber, a higher result, say, one above 17%, would have been puzzling.

Apparent Molecular Weight of Rubber

The ratio of the total number of chain carbon atoms to the total number of junction points is 432, and as there are two chains passing through every junction point, each freely swinging arc has 216 chain carbon atoms, when x is 850%. Knowing that there are very many of these arcs in the single molecular chain, one sees in the mathematics a confirmation of the generally accepted view that rubber has a very high molecular weight.

This result also shows that by far the greater proportion of the internal structure of soft vulcanized rubber has the properties of a liquid and that only $\frac{2}{432} \times 100 = 0.46\%$ of its chain carbon atoms exist in a solid phase.

The great length of the molecular arc makes complete rotation around an axis impossible. The rotation under consideration must, therefore, be of an oscillatory or vibratory kind, with consequent twisting or deformation of the arc.

Equation Relating Stress and Strain

λ, the half-length, in centimetres, of the molecular chain between adjacent junction points, may be calculated from the fact that it becomes equal to ξ at maximum elongation.

F, the stress in kilograms per square centimetre, is obtained by multiplying A, the horizontal component of the tension in a single chain, by the number of chains per square centimetre, and converting the units from dynes to kilograms. The determination of the percentage elongation, ϵ, is also a simple arithmetical calculation from X-ray data, ξ and λ, and is given later. And now, knowing F and ϵ in terms of the variables used in the equation for η, the energy in the single arc, the following equation relating stress and elongation can be written—

$$F = \frac{\eta}{4.20 \times 10^{-16} C(\epsilon+100)}, \tag{26}$$

where C and ϵ are interrelated, C being obtainable by reference to the tables of elliptic functions. Curve B, Fig. 8, is drawn from this equation.

η cannot be calculated from atomic data, and consequently its value has been ascertained by supplying in the above equation known values of F and ϵ from the experimental curve. It is the only constant obtained in this way.

One reason why η is assumed to remain constant during stretching is that the atoms whose rotational motion resists the applied stress are moving virtually at right angles to the direction of the stress. Such being the case the stress has no effect upon their rotational motion or rotational energy. Thus the energy stored in the rotating arc by the separation of junction points is essentially potential in nature and not kinetic, and does not affect the value of η.

On rapid stretching the energy created by simplification of structure has no chance to become dissipated into the atmosphere. A stiffer stress-strain curve should result therefore, from this cause as well as from the fact that there is less time for plastic flow. On slow stretching, where there is time for the elimination of heat, however, η should remain more or less constant, and as the rate of stretching for the experimental Curve A, Fig. 8, was at the slow rate of 20 in. per minute, η is assumed constant in the present calculation.

Elastic Energy in Rubber

The value of η was found to be 7.12×10^{-12} ergs. η represents the energy in a complete chain, or 216 chain carbon atoms. Knowing the total number of chain carbon atoms in 1 cc., it is easy to calculate the total energy of rotation in 1 cc., which is 9.94×10^{8} ergs, or 24 gram calories. Assuming an approximate heat capacity of 0.47 cal. per cc. per degree (42, p. 872), this value is approximately equivalent to the amount of heat necessary to raise the temperature of the rubber 51° C. and, very roughly,

$$\frac{51}{293} \times 100 = 17\% \tag{31}$$

of the heat necessary to raise the temperature of the rubber from absolute zero to room temperature. Therefore, very roughly, 17% of the total heat energy in rubber is used in producing elasticity.

Although the above constant is only approximate, and necessarily depends on the correctness of the assumptions, nevertheless one is struck with the enormous possibilities of valuable information concerning the structure of rubber which can result from the application of mathematics to the problem.

Agreement of Stress-Strain Equation with Joule Effect

It is evident from Equation (26) that an increase in η, due to rise in temperature, would increase F for a given value of ϵ, C remaining constant when ϵ is constant. This explains why a specimen held extended under a constant load contracts when heated. The equation is thus in accord with the Joule effect. F, representing the internal stresses in the specimen resisting deformation, opposes, and is in equilibrium with, the constant load. An increase in F would result in a differential force causing the specimen to contract, and the contraction would continue until F, which naturally decreases with the contraction of the rubber, became equal to the constant load. Conversely, cooling would cause an increase in the elongation of the specimen under constant load.

Further, as η should be proportional to the temperature of the rubber, if ϵ is kept constant, making C constant, F for a given elongation should be a straight line function of temperature, provided that plasticity in the rubber can be eliminated.

The S-shape of the Stress-Strain Curve

Curve B, Fig. 8, has been drawn by means of Equation (26). It will be noticed that F becomes zero only when ϵ becomes equal to -100%. The significance of this is that, mathematically, the force of attraction between two adjacent junction points becomes zero only when the distance between them becomes zero, which is the case when ϵ equals -100%, whereas actually in rubber there is a definite distance between junction points, equal to $2\xi_0$, at zero stress. This is due to the fact that the junction points themselves repel one another owing to heat vibrations transmitted through the structure when they are close together and this repulsion balances the attraction between them at 0% elongation.

An attempt to formulate an equation passing through the origin has been made as follows. It is assumed that when junction points separate along the line of the stress they approach one another in a direction at right angles to the stress, because, the volume of the rubber remaining practically constant during stretching, the junction points must occupy a constant volume. Now, as the structure of the rubber is, as previously mentioned, liquid in nature, pressures in this medium are transmitted in all directions. Thus will the attraction between junction points at right angles to the direction of the external stress, owing to rotation, be transmitted as a sort of liquid pressure to the direction parallel to the stress, and tend to separate the junction points in the latter direction, thereby assisting the deforming stress. At 0% elongation, where the junction points are equidistant, there is an equality of junction point pressure in all directions, just balancing the attraction due to rotation,

and the resultant force on the junction point is zero. However, when the orientation is disturbed by deformation there is a greater force attracting the junction points in the external stress direction than at right angles to the stress, and the resultant force resisting deformation is the difference between the attraction, parallel to the stress, between junction points, due to rotation, and the attraction due to rotation at right angles to the stress. An equation to represent this condition has been developed as follows:

$$F_r = F_l - F_c = \frac{\eta}{4.20 \times 10^{-16}\, C_l(\epsilon_l + 100)} - \frac{\eta}{4.20 \times 10^{-16}\, C_c(\epsilon_c + 100)} \qquad (28)$$

where

F_l is the stress in kilograms per square centimetre caused by junction point attraction parallel to the external stress,

F_c is the stress in kilograms per square centimetre caused by junction point attraction at right angles to the external stress,

F_r is the resultant stress resisting deformation, equal to $F_l - F_c$,

ϵ_l is the percentage elongation of the specimen, parallel to the stress, and therefore the elongation used in drawing the stress-strain curves,

ϵ_c is the percentage elongation of the specimen, at right angles to the stress. Naturally, as ϵ_l increases ϵ_c decreases and is always less than 0%. ϵ_c is calculated from ϵ_l from the fact that the junction points always occupy a constant volume.

C_l is the elliptic function corresponding to, and depending on, ϵ_l,

C_c is the elliptic function corresponding to, and depending on, ϵ_c.

Curve C, Fig. 7, has been drawn from this equation. It will be noticed that the stress becomes zero when the elongation becomes zero but, unlike the experimental curve, it has no S-shape.

As the junction points move farther apart in the direction of stretch they approach one another at right angles to this direction. A calculation was made to determine at what elongation the junction points came within the distances between chains, measured transversely to the stress, shown in the Meyer and Mark X-ray diagram, Fig. 7. It was found that this occurred at 1960%, indicating that at no time, even at the ultimate elongation, do the junction points approach very close to one another and that there are always a number of chain molecules between them. It is not likely that the liquid pressure mentioned above could be transmitted from junction point to junction point with a large number of chains intervening, and as the junction points move farther apart in the direction of stretch, the effect of transverse junction point pressure in assisting the stress would gradually disappear. The second part of Equation (28) would then become negligible and this equation would become similar to Equation (26). Thus there is a point where the curve of Equation (28) should merge into the curve of Equation (26), giving an S-shape.

It is difficult, in view of the fact that the Schopper machine on which the experimental sample was tested is not very accurate at low elongations, to

state just where the merging occurs. This point, however, has been determined experimentally for a pure gum compound by Shacklock (32), who finds "the inflexion point" at the "correct" cure to be at 72% elongation, and one concludes that the second part of Equation (28) disappears above 72% elongation, or long before any considerable relative displacement of junction points takes place.

Another way of considering this phenomenon, and possibly a better one, is to suppose the rubber specimen to be contracting. During the contraction, above 72%, Curve B is followed. Then at 72% the retracting junction points begin to get within the range of one anothers' influence, and begin to push one another apart at right angles to the external stress, thus decreasing their tendency to retract in the direction of the stress, so that Curve B, instead of continuing down to -100% elongation, becomes deflected to the point of zero stress and zero elongation. The experimental Curve A, if continued down to -100% elongation would lose all trace of an S-shape. This is true not only of Curve A, but of all experimental stress-strain curves for rubber, however compounded.

Hatschek (16) stated that by correcting for the diminution in area of cross section of the test piece, during extension, the point of inflexion disappeared. Shacklock (32), who made an accurate study of the stress-strain curve at low elongations, finds that the S-shape is still noticeable even when the stress is expressed as the force per unit of actual cross section.

It is not difficult to understand why the S-shape should become less apparent when the correction for the diminution in area of cross section is made. As the test piece becomes stretched the cross section diminishes, and the load, corrected for actual cross section, is thus greater than it would be were it calculated on a constant unit cross section. The calculated Curve B, Fig. 8, for example, if corrected, would cross the uncorrected Curve B at 0% elongation and lie closer to the elongation axis between -100% and 0% elongation, and closer to the stress axis above 0% elongation, than the uncorrected curve, *i.e.*, the slope to the stress axis above 0% elongation would decrease. Now, as already stated, the actual stress-strain curve is one which starts at zero elongation and zero stress and joins on to the calculated curve at around 72% elongation. It is apparent that a curve starting from the origin would merge the less abruptly into the calculated curve the less its slope at the point of merging. Thus any factor which decreases the slope of the stress-strain curve to the stress axis, such as correcting for actual cross section, or, for that matter, the addition of stiffeners to the rubber compound, will tend to make the S-shape less noticeable. This statement receives ample verification in actual experiment and it is well known that the S-shape is more apparent in the stress-strain curves of pure gum compounds than in heavily loaded compounds.

Thus the mathematics evolves from a well known theory of rubber structure a feasible explanation of the peculiar S-shape of the stress-strain curve for

rubber and shows why this S-shape becomes less apparent when the calculation of the stress is based on the actual cross section.

Means of Improving the Accuracy of the Calculated Stress-Strain Curve

In spite of the fact that Curves A and B have been made to agree as closely as possible at infinite stress and to coincide at 158% elongation (the value of η having been calculated from the value of F at the latter elongation), the calculated curve is stiffer than the experimental. In other words, in rubber there is a greater elongation produced by a given stress than can be calculated from rotation alone. This may be taken as strong evidence that there is a wave motion along the chain, and that the additional elongation caused by a given stress in the experimental curve is due to the unwinding of the wave motion along the molecular chains, which takes place along with the separation of the junction points.

The incorporation of wave motion into the equation relating stress and strain would take the form of a correction in the elongation. First of all, x, the elongation at infinite stress, must be reduced so that when the addition is made to the elongation to take care of wave motion, the calculated curve will coincide with the experimental at high elongation. Corrections will also have to be made all along the curve. While these corrections may easily be made empirically, an attempt to find a theoretical basis for them would undoubtedly be better.

The experimental Curve A, Fig. 8, is the curve obtained during the first stretching of the rubber specimen. It has long been known (8) that if rubber is stretched to a point fairly close to its breaking elongation and then allowed to retract, a further stretching yields a stress-strain curve closer to the elongation axis than the first one. The stress-strain curve for the next stretching is still closer to the elongation axis, and so on until a point is reached where only relatively slight changes in the position of the stress-strain curve are produced by further stretching. As the structure of fatigued rubber has greater permanency than that of unstretched rubber, it might be thought advisable to adopt the stress-strain curve of fatigued rubber or the reversible curves of Williams (42) or Gerke (14) instead of Curve A in the present instance. The objection to this procedure, however, is that continued stretching leaves the rubber in an anisotropic condition, owing to plastic flow, or other reasons. As in the mathematics a perfectly regular orientation of the structure is assumed, with the junction points ranged evenly along the three axes of reference, it was considered better to adopt the stress-strain curve produced by the first stretching as being more typical of isotropic rubber.

In the mathematics the network is assumed to be oriented along three axes at right angles to one another, one of the axes being parallel to the stress, which is actually, of course, not the case. However, as the value of η was obtained by supplying experimental values of F and ϵ in the equation relating stress and strain, η becomes the energy of arc which would give the above

values of F and ϵ, assuming a regularly oriented structure. The more exact calculation of η would encumber the mathematics considerably, and, in any case, such a procedure is quite unnecessary in a first approximation.

It is obvious, in view of the fact that vulcanized hard and soft rubber undergo plastic flow under stress at ordinary and at elevated temperatures, that the sulphur cross linkages are not unbreakable and that they can shift their position, giving permanent set. As vulcanized rubber, in common with crude rubber and other elastic polymers, exhibits plasticity, there is apparently no essential difference, except one of degree, among all elastic polymers. The chief reason for the superiority of vulcanized rubber is probably in a somewhat tighter, though still plastic, cross linkage bond, situated at the sulphur atom. Whitby (39) has expressed the view that the essential process in the development of elasticity in all elastic polymers, including vulcanized rubber, is a polymerization. This view is in no way opposed to the theory brought forward in this paper, where an attempt is made to show on physical grounds what kind of polymerization exists in vulcanized rubber, and to present a picture of its structure.

In making further corrections to the calculated stress-strain curve the effect of the change in structure actually taking place during the stretching, *i.e.*, the effect of such factors as plastic flow and breaking up of junction points should be considered as well as the effect of wave motion.

The expressions which have been worked out for the constants being of a general nature, the numerical values of x, s, r and c not having been filled in, these constants may be corrected as soon as a more accurate value for x has been obtained.

As the discrepancy between the calculated and experimental curves does not exceed 65% on the elongation axis at any point, it is seen that the errors in the constants caused by the neglect of wave motion and plastic flow in the calculations are not of serious proportions and not sufficient to alter any conclusions which have been drawn from the present results. In addition to this it has been shown that the rotation theory leaves a stress-strain curve closely approximating that for rubber, and it is doubtful whether any of the other existing theories would stand up as well if it were possible to work out their mathematical consequences.

Mathematical

The following development of a formula for the skipping rope curve is taken from a treatise on elliptic functions by Appell and Lacour (1, p. 207-211).

The rotating part of the rubber molecule, which is assumed for present purposes to be homogeneous along its length, will be on a curve in which there is equilibrium between centrifugal force and tension in the molecule.

The centrifugal force acting outwards on an element of mass, m, is perpendicular to the axis of rotation. Its intensity is $m\omega^2 r$, ω representing the

angular velocity of rotation and r the distance of the element, m, from the axis. Taking Ox (Fig. 3) as the axis of rotation, the problem may be briefly stated as follows.

A cord is attached at two points to the axis Ox and each element of the cord is repelled by the axis by a force proportional to the length of the element and to its distance from the axis.

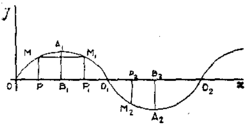

FIG. 3. *Skipping rope curve.*

As all the forces acting on the cord meet the axis Ox, the moment of the tension about this axis is constant throughout its length but, as the cord is attached to the axis at two points, the moment of the tension at the ends is zero. This moment is, therefore, zero throughout the axis, and introducing the z axis to take care of the general case where the arc is not in the vertical plane, the following equation for the moment is obtained:

$$T\left(y\frac{dz}{ds} - z\frac{dy}{ds}\right) = 0\,,$$

whence

$$\frac{dy}{y} - \frac{dz}{z} = 0\,, \quad y = mz\,,$$

m being a constant. The equilibrium diagram is, therefore, in a plane passing through the axis. Let us call this the xy plane, the force per unit length, Y, acting on the element, ds, being perpendicular to Ox, repulsive and proportional to the y ordinate. Thus

$$Yds = \mu y ds\,,$$

where μ is a constant and equal to $q\omega^2$, q being the mass per unit length of the molecule and ω the angular velocity.

The equations of equilibrium are, then,

$$d\left(T\frac{dx}{ds}\right) = 0\,, \qquad d\left(T\frac{dy}{ds}\right) + \mu y ds = 0\,,$$

he first giving

$$T\frac{dx}{ds} = A\,,$$

where one can always suppose A positive by computing the arc length, s, in uch a way that x increases with s. A is the horizontal component of the ension, T. Using this value of T in the second equation and making

$$\frac{dy}{dx} = y', \frac{\mu}{A} = \frac{2}{a^2}\,, \quad ds = \sqrt{1+(y')^2} = \frac{dy\sqrt{1+(y')^2}}{y'}\,,$$

e following equation is obtained:

$$\frac{y'dy'}{\sqrt{1+(y')^2}} + \frac{2ydy}{a^2} = 0\,,$$

d, on integrating,

$$\sqrt{1+(y')^2} + \frac{y^2}{a^2} = \frac{b^2}{a^2}\,,$$

b^2 representing a constant necessarily positive since the first member is positive. Isolating the radical, squaring, and substituting for y' its value, $\frac{dy}{dx}$, gives:

$$dx = \pm \frac{a^2 dy}{\sqrt{(b^2-y^2)^2-a^4}}. \tag{P}$$

As the rope is attached to the axis, Ox, the equation should give a real value for y' when $y=O$; whence $b^2 > a^2$. Designating by $\phi(y)$ the polynomial under the radical,

$$\phi(y) = (b^2+a^2-y^2)\ (b^2-a^2-y^2)\ .$$

y, starting from zero, can vary only between $-\sqrt{b^2-a^2}$ and $+\sqrt{b^2-a^2}$. In the construction of the curve let it be supposed that the cord is attached at O (Fig. 3) and that it is situated in the angle yOx; then x increases with y, $\frac{dy}{dx}$ is positive, and

$$x = \int_0^y \frac{a^2 dy}{\sqrt{\phi(y)}}; \tag{Q}$$

while y increases, x increases, until $y = \sqrt{b^2-a^2}$; x then has the value:

$$\xi = \int_0^{\sqrt{b^2-a^2}} \frac{a^2 dy}{\sqrt{\phi(y)}}. \tag{R}$$

The branch OA_1 is thus obtained, the tangent at A_1 being horizontal. Then y decreases, and in order that x may continue to increase, it is necessary to put the minus sign in front of $\sqrt{\phi(y)}$. Thus there is obtained a new branch, $A_1M_1O_1$, symmetrical to the first, OMA_1, with respect to the ordinate, A_1B_1, because equal variations in x correspond to equal variations in y. At the point O_1, $y=O$, and the abscissa is 2ξ. Then y, becoming negative, decreases until it reaches the value $-\sqrt{b^2-a^2}$; the abscissa increases again to the value 3ξ, which gives the point A_2, where the tangent is horizontal. Then y increases again from $-\sqrt{b^2-a^2}$ to $+\sqrt{b^2-a^2}$. After the value A_2 it is necessary to put the plus sign before the radical, the arc $A_2O_2A_3$ thus being obtained, cutting the axis at the point O_2 where the abscissa is 4ξ, and so on. The curve branches thus obtained are successively all equal to the first. The curve is thus analogous to a sinusoidal curve.

The following substitutions may now be made in Equation (Q):

$$y = t\sqrt{b^2-a^2},\ k^2 = \frac{b^2-a^2}{b^2+a^2} < 1\ ,\quad 1-k^2 = (k')^2 = \frac{2a^2}{b^2+a^2}\ . \tag{1}$$

Equation (Q) now takes the form:

$$\frac{x\sqrt{2}}{ak'} = \int_0^t \frac{dt}{\sqrt{(1-t^2)\ (1-k^2t^2)}}\ .$$

As this equation now has the form of Legendre's standard elliptic integral of the first kind, it may be represented, by the customary notation, as follows:

$$t = \operatorname{sn}\frac{x\sqrt{2}}{ak'}, \quad y = \sqrt{b^2-a^2}\operatorname{sn}\frac{x\sqrt{2}}{ak'}.$$

Thus the curve gives a graphical representation of the variation of the sn function.

The differential, ds, of the arc of the curve is

$$ds = \sqrt{1+\left(\frac{dx}{dy}\right)^2}\,.\,dy.$$

By substituting in this equation the value for $\frac{dx}{dy}$ obtained in Equation (P),

$$ds = \frac{(b^2-y^2)\,dy}{\sqrt{\phi(y)}},$$

and

$$s = \int_0^y \frac{(b^2-y^2)\,dy}{\sqrt{\phi(y)}}. \tag{S}$$

By making Substitution (1) in Equations (Q) and (S) there is found for abscissa ξ at the point A_1, and the length, λ, of the arc OA_1, the two expressions:

$$\left.\begin{aligned} \xi &= \frac{ak'}{\sqrt{2}}\int_0^1 \frac{dt}{\sqrt{(1-t^2)(1-k^2t^2)}} = \frac{ak'}{\sqrt{2}}K, \\ \lambda &= \frac{a}{k'\sqrt{2}}\int_0^1 \frac{(1+k^2-2k^2t^2)\,dt}{\sqrt{(1-t^2)(1-k^2t^2)}}, \end{aligned}\right\} \tag{2}$$

because the point A_1 is obtained by making $t=1$, or $y=\sqrt{b^2-a^2}$ in Substitution (1).

All the elliptic functions developed in the present work can be reduced to terms of the elliptic symbols K and E.

$$K = \int_0^1 \frac{dt}{\sqrt{(1-t^2)(1-k^2t^2)}}, \quad \text{or, if } t = \sin\phi,$$

$$K = \int_0^{\frac{\pi}{2}} \frac{d\phi}{\sqrt{1-k^2\sin^2\phi}}.$$

$$E = \int_0^1 \sqrt{\frac{1-k^2t^2}{1-t^2}}\,.\,dt, \text{ or, if } t = \sin\phi,$$

$$E = \int_0^{\frac{\pi}{2}} \sqrt{1-k^2\sin^2\phi}\,.\,d\phi.$$

In the tables of elliptic functions (29, 33) the values of K and E are given for the corresponding values of $\sin^{-1} k$.

When λ and ξ are given (λ being greater than ξ because the arc OA_1 is greater than its projection OB_1) the constants a and k^2 have only one system of values, under the condition $k^2 < 1$. In fact, by calculating $\lambda - \xi$ and $\lambda + \xi$ the following relation is obtained:

$$\frac{\lambda - \xi}{\lambda + \xi} = k^2 \frac{\int_0^1 \sqrt{\frac{1-t^2}{1-k^2t^2}} \cdot dt}{\int_0^1 \sqrt{\frac{1-k^2t^2}{1-t^2}} \cdot dt} \quad . \tag{3}$$

For $k^2 = 0$, the value of the second member is zero. As k^2 increases, the numerator obviously increases and the denominator decreases. Thus the ratio increases, and for $k^2 = 1$ the ratio is 1. This ratio, then, passes once and only once through the given value $\frac{\lambda - \xi}{\lambda + \xi}$. The constant k^2 has, therefore, one and only one value. Equation (2) for ξ thus gives a single value for a.

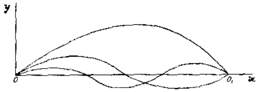

FIG. 4. *Waves in skipping rope curve.*

There are an infinite number of positions of equilibrium for the cord, assuming it to have a given length, l, and to be attached at the points, O and O_1, to the axis, Ox, Fig. 4. Let the length, OO_1, be α.

1. The cord has only a single wave between O and O_1. Then ξ is half of α, λ half of l. The quantities ξ and λ being known, the constant, k^2, has a single value given by Equation (3), and $a = \frac{\alpha\sqrt{2}}{2Kk^1}$.

2. The cord has two waves between O and O_1. Then $\xi = \frac{\alpha}{4}$, $\lambda = \frac{l}{4}$. k^2 has the same value as in the preceding case because $\frac{\lambda - \xi}{\lambda + \xi}$ has the same value, $\frac{l-\alpha}{l+\alpha}$, and $a = \frac{\alpha\sqrt{2}}{4Kk^1}$.

In general, if the cord has n waves between O and O_1, $\xi = \frac{\alpha}{2n}$, $\lambda = \frac{l}{2n}$; k^2 has always the same value, but $a = \frac{\alpha\sqrt{2}}{2nKk^1}$. There is, therefore, an infinity of positions of equilibrium, all of which pass through O and O_1. In the assumed structure for rubber there is only one wave between O and O_1.

In order to express stress in terms of strain in rubber it is necessary to find a relation between A and ξ. These quantities are brought together in the equation for the energy of rotation, which is developed, as follows, in a manner similar to that given by Appell and Lacour (1, p. 207-211).

The kinetic energy due to the velocity of rotation of a particle of length, ds, in the skipping rope is represented by $\frac{q\,ds\,\omega^2 y^2}{2}$. In this case, y, the distance of the particle, ds, from the axis Ox is the radius of rotation. ω, as before, is the angular velocity of rotation and q the mass per unit length.

The energy in the length of rope from O to A_1, Fig. 3, *i.e.*, in the length, λ, equals:

$$\frac{q\omega^2}{2}\int_0^{\sqrt{b^2-a^2}} y^2 ds\ .$$

As

$$ds = \sqrt{1+\left(\frac{dx}{dy}\right)^2}\,dy = \frac{(b^2-y^2)dy}{\sqrt{\phi(y)}},$$

then

$$y^2 ds = \frac{y^2(b^2-y^2)dy}{\sqrt{\phi(y)}};$$

therefore, the kinetic energy in the complete arc, OA_1O_1, *i.e.*, in the length, 2λ, equals

$$\eta = q\omega^2\int_0^{\sqrt{b^2-a^2}} \frac{y^2(b^2-y^2)dy}{\sqrt{\phi(y)}}.$$

Substitution (1) gives:

$$\eta = \frac{\sqrt{2}q\omega^2a^3k^2}{(k')^3}\int_0^1 \frac{(1+k^2-2k^2t^2)t^2dt}{\sqrt{(1-t^2)(1-k^2t^2)}}\ . \tag{4}$$

The integral in Equation (4) may be simplified by expansion.

$$\int_0^1 \frac{(1+k^2-2k^2t^2)t^2dt}{\sqrt{(1-t^2)(1-k^2t^2)}} = (1+k^2)\int_0^1 \frac{t^2dt}{\sqrt{(1-t^2)(1-k^2t^2)}} - 2k^2\int_0^1 \frac{t^4dt}{\sqrt{(1-t^2)(1-k^2t^2)}}\ .$$

The problem is to express Equations (3) and (4) in terms of K and E so that the tables may be used in their evaluation. Starting with Equation (3), letting $t = \sin\phi$, and simplifying by expanding into partial fractions,

$$\frac{\lambda-\xi}{\lambda+\xi} = k^2\frac{\int_0^1\sqrt{\frac{1-t^2}{1-k^2t^2}}\ .\ dt}{\int_0^1\sqrt{\frac{1-k^2t^2}{1-t^2}}\ .\ dt} = k^2\frac{\int_0^{\frac{\pi}{2}}\frac{(1-\sin^2\phi)d\phi}{\sqrt{1-k^2\sin^2\phi}}}{\int_0^{\frac{\pi}{2}}\sqrt{1-k^2\sin^2\phi}\ .\ d\phi}$$

$$= k^2\frac{\int_0^{\frac{\pi}{2}}\frac{d\phi}{\sqrt{1-k^2\sin^2\phi}} - \int_0^{\frac{\pi}{2}}\frac{\sin^2\phi\ .\ d\phi}{\sqrt{1-k^2\sin^2\phi}}}{\int_0^{\frac{\pi}{2}}\sqrt{1-k^2\sin^2\phi}\ .\ d\phi}.$$

Now,

$$\int_0^{\frac{\pi}{2}}\frac{\sin^2\phi\ .\ d\phi}{\sqrt{1-k^2\sin^2\phi}}$$

may be simplified by expansion (29) to

$$\frac{1}{k^2}\int_0^{\frac{\pi}{2}}\frac{d\phi}{\sqrt{1-k^2\sin^2\phi}}-\frac{1}{k^2}\int_0^{\frac{\pi}{2}}\sqrt{1-k^2\sin^2\phi}\,.\,d\phi$$

and this equals

$$\frac{K}{k^2}-\frac{E}{k^2}\,.$$

Therefore,

$$\frac{\lambda-\xi}{\lambda+\xi}=\frac{Kk^2-K-E}{E}=B\,. \tag{9}$$

By making $t=\sin\phi$ in the first partial fraction in Equation (4),

$$\int_0^1\frac{t^2dt}{\sqrt{(1-t^2)(1-k^2t^2)}}=\int_0^{\frac{\pi}{2}}\frac{\sin^2\phi\,.\,d\phi}{\sqrt{1-k^2\sin^2\phi}}\,.$$

This, as has been shown in the evaluation of Equation (3), equals

$$\frac{K}{k^2}-\frac{E}{k^2}.$$

In the evaluation of the second partial fraction in Equation (4) the standard notation of Legendre is used. Let

$$\int_0^t\frac{dt}{\sqrt{(1-t^2)(1-k^2t^2)}}=u.$$

Then, if $t=\sin\phi$, $t=\operatorname{sn} u$. By using the method of elliptic functions, the following equalities may be derived by the substitution of sn u for t:

$$\int_0^1\frac{t^2dt}{\sqrt{(1-t^2)(1-k^2t^2)}}=\int_0^K\operatorname{sn}^2u\,du=\frac{K}{k^2}-\frac{E}{k^2},$$

$$\int_0^1\frac{t^4dt}{\sqrt{(1-t^2)(1-k^2t^2)}}=\int_0^K\operatorname{sn}^4u\,du.$$

For the evaluation of the integral, $\int_0^K \operatorname{sn}^4u\,du$ Appell and Lacour (1, p. 276-277) use an expansion of the expression, $\operatorname{sn}^{2n-3}u\operatorname{cn}u\operatorname{dn}u$, obtained by differentiating it according to the ordinary methods of calculus and then integrating, as follows:

$$\frac{d}{du}\left[\operatorname{sn}^{2n-3}u\operatorname{cn}u\operatorname{dn}u\right]=(2n-3)\operatorname{sn}^{2n-4}u\,(1-\operatorname{sn}^2u)\,(1-k^2\operatorname{sn}^2u)-\operatorname{sn}^{2n-2}u\,(1-k^2\operatorname{sn}^2u)-k^2\operatorname{sn}^{2n-2}u(1-\operatorname{sn}^2u),$$

or, on simplifying,

$$(2n-3)\operatorname{sn}^{2n-4}u-(2n-2)\,(1+k^2)\operatorname{sn}^{2n-2}u+(2n-1)\,k^2\operatorname{sn}^{2n}u.$$

On integrating,

$$\operatorname{sn}^{2n-3}u\operatorname{cn}u\operatorname{dn}u=(2n-3)\int\operatorname{sn}^{2n-4}u\,du-(2n-2)\,(1+k^2)\int\operatorname{sn}^{2n-2}u\,du+(2n-1)k^2\int\operatorname{sn}^{2n}u\,du.$$

Putting $n=2$ in this equation,

$$\operatorname{sn} u \operatorname{cn} u \operatorname{dn} u = \int du - 2(1+k^2)\int \operatorname{sn}^2 u\, du + 3k^2 \int \operatorname{sn}^4 u\, du \quad ,$$

whence

$$\int_O^K \operatorname{sn}^4 u\, du = \frac{1}{3k^2}\left(\int_O^K \Big[\operatorname{sn} u \operatorname{cn} u \operatorname{dn} u\Big] - \int_O^K du + 2(1+k^2)\int_O^K \operatorname{sn}^2 u\, du\right)$$

$$= \frac{1}{3k^2}\left(O - K + 2(1+k^2)\left[\frac{K}{k^2} - \frac{E}{k^2}\right]\right) \;.$$

The value of η can now be expressed in terms of K, E and k, as follows:

$$\eta = \frac{\sqrt{2}q\omega^2a^3k^2}{(k')^3}\left(\frac{K+E}{3} - \frac{K-E}{3k^2}\right) \;.$$

The substitutions

$$q\omega^2 = \mu \;, \quad a^2 = \frac{2A}{\mu} \;, \quad a = \frac{\xi\sqrt{2}}{Kk'} \text{ (from Equation (2))} \;, \quad a^3 = \frac{2\sqrt{2}A\xi}{\mu Kk'} \quad ,$$

are useful in simplifying the equation for η.

$$\eta = \frac{4A\xi k^2}{K(k')^4}\left(\frac{K+E}{3} - \frac{K-E}{3k^2}\right) \;.$$

Letting

$$\frac{k^2}{K(k')^4}\left(\frac{K+E}{3} - \frac{K-E}{3k^2}\right) = C \quad ,$$

$$\eta = 4A\xi C \;. \tag{10}$$

The next step is to find a means of expressing A in terms of the stress on the rubber specimen in kilograms per square centimetre and ξ in terms of the percentage elongation.

Calculation of n

The present theory assumes the rubber structure to be made up entirely of swinging arcs in irregular arrangement. The arrangement is such that the arcs do not rotate in pairs on opposite sides of the same axis, although some of the arcs might take up this position. It is more probable that the adjacent junction points about which one arc rotates are formed by the union of three separate molecular chains. Thus there are three separate molecular chains for each pair of junction points, and the calculation is based on this type of structure.

In the calculation of the total number of junction points in rubber, they are supposed to be aligned along three co-ordinates parallel to the edges of the cube. While the structure has, strictly speaking, not this extreme regularity, the assumption is necessary for the mathematical development, the results justifying it.

The next step is to suppose the cube of rubber to be stretched along the three co-ordinates until the molecular arcs are perfectly aligned and lie evenly between their respective junction points. Fig. 5 shows a cubic lattice illustrating the transformed rubber structure, the lines representing molecular chains and the points where they meet, the junction points.

The degree of stretch along the three co-ordinates necessary to attain perfect alignment of molecular chains is taken as x, the percentage elongation represented by the asymptote, LM, to the experimental stress-strain curve (see Curve A, Fig. 8). This is called the elongation at infinite stress, a constant which figures considerably in the mathematical work.

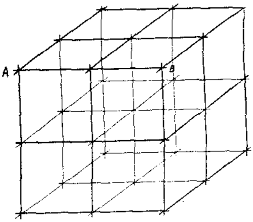

FIG. 5. *Cubic lattice used in calculations.*

The number of chain ends along any one of the edges, such as AB, of the cube in Fig. 5, being n, the total number of chains is $3n^2$ and the total number of junction points, n^3. However, as it is impossible for three chains to be joined together at one point, Fig. 6 gives a better idea of the true appearance of the rubber structure, where there are actually $2n^3$ junction points, there being two junction points now for every one represented in Fig. 5. The total number of chains remains the same, namely $3n^2$.

The reasoning prompting the drawing of Fig. 6 is based upon the assumption that it is impossible for one chain molecule to pass permanently into the territory of oscillation of another chain by crossing it at a sharp angle without forming a junction point with it at the point where they cross. Again, it is not possible for there to be more junction points than represented in Fig. 6, because the incorporation of more junction points into the figure than already shown would necessitate a reduction in x. Thus the mathematical artifice used for the calculation of the total number of junction points per cubic centimetre gives the maximum number of junction points for an elongation of x per cent at infinite stress. Fewer junction points than this would mean that the forces of attraction between molecular chains failed to act at points where they would be most likely to act (*i.e.*, where the chains crossed), which is improbable. The basis of the calculation, therefore, in addition to being justified by the results obtained, is in itself not unreasonable.

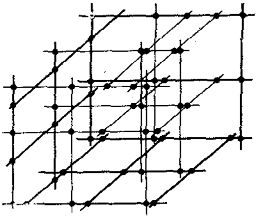
FIG. 6. *Postulated structure of distended rubber, showing junction points.*

In the calculation of the total number of junction points on one chain (each chain assumed to extend from one side of the cube to the opposite side), it must be borne in mind that each junction point exists on two chains. There are $2n^3$ junction points, each affecting two chains, and there are $3n^2$ chains. Thus the total number of junction points on one chain equals

$$\frac{2 \times 2n^3}{3n^2} = \frac{4n}{3}.$$

As previously stated, 1 cc. of rubber compound contains $src \times 10^{-4}$ gm. of pure rubber. The molecular weight of the C_5H_8 unit being 68.06, and the mass of the hydrogen atom 1.66×10^{-24} gm. the actual weight of a C_5H_8 unit is

$$\frac{68.06 \times 1.66 \times 10^{-24}}{1.008} \text{ gm.}$$

The number of C_5H_8 units in 1 cc. of rubber compound is, therefore, the weight of pure rubber in 1 cc. of compound divided by the weight of a C_5H_8 unit, or

$$\frac{src \times 10^{-4} \times 1.008}{68.06 \times 1.66 \times 10^{-24}} = 8.92 \times 10^{17}\, src.$$

As one of the carbon atoms in isoprene is on a side chain, there are only four chain-forming carbon atoms per isoprene unit. Therefore, the number of chain-forming carbon atoms in 1 cc. of rubber compound is

$$4 \times 8.92 \times 10^{17}\, src = 3.57 \times 10^{18}\, src. \qquad (11)$$

The length of 1 cc. of rubber after being stretched $x\%$ will be $\frac{(x+100)}{100}$ cm. Assuming the rubber to be stretched to this extent along three axes at right angles to one another, all the chains will be perfectly aligned along the three axes, as illustrated in Fig. 6. The distance between carbon atoms along the chain is best obtained by the use of X-ray data. In Fig. 7, due to Meyer and Mark (26), it will be observed that the chains are aligned along the c-axis of the unit cell and that there are eight carbon atoms in the cell distance of 8.1×10^{-8} cm. The distance between neighboring carbon atoms along the chain is, therefore,

$$\frac{8.1 \times 10^{-8}}{8} = 1.01 \times 10^{-8} \text{ cm.}$$

The total number of carbon atoms in a complete chain, *i.e.*, in a distance of $\frac{(x+100)}{100}$ cm. is $\frac{(x + 100)}{100 \times 1.01 \times 10^{-8}} = \frac{(x + 100)}{1.01 \times 10^{-6}}$.

The division of the total number of chain carbon atoms by the item just obtained gives the total number of chains in 1 cc., each chain being equal in length to the distance between opposite faces of the distended cube.

$$\frac{3.57 \times 10^{18}\, src \times 1.01 \times 10^{-6}}{(x + 100)} = \frac{3.61 \times 10^{12}\, src}{(x + 100)}.$$

This figure is the total number of chains in 1 cc., and as the chains are assumed to be aligned parallel to the three axes, the number of chains aligned parallel to any one axis is one-third of this figure, or

$$\frac{1.20 \times 10^{12}\, src}{(x + 100)}. \qquad (13)$$

The number of chain ends along any one of the edges of the cube, or n, is the square root of item (13).

$$n = \sqrt{\frac{1.20 \times 10^{12}\, src}{(x + 100)}} = 1.10 \times 10^{6} \sqrt{\frac{src}{(x + 100)}}. \qquad (14)$$

Calculation of ξ_o

The number of junction points on each chain is, as previously calculated, $\frac{4n}{3}$, or

$$1.46 \times 10^{6} \sqrt{\frac{src}{(x + 100)}} . \qquad (15)$$

The number of junction points per chain was calculated by postulating an imaginary condition of the rubber where a cube is distended to the geometrical limit. The present theory assumes that there are as many junction points in rubber in the natural, unstretched state as there are in this imaginary distended state. In the normal state, since the calculations are being made on 1 cc. of rubber compound, the distance between chain ends is 1 cm., and for the purposes of the calculation the junction points are still assumed to lie on lines parallel to three co-ordinates at right angles to one another and parallel to the sides of the cube. The distance, therefore, between adjacent junction points on the same chain when the rubber is at 0% elongation is the reciprocal of item (15), and equals

$$2\xi_o = \frac{1}{1.46} \times 10^{-6} \sqrt{\frac{(x + 100)}{src}} ;$$

$$\xi_o = 3.42 \times 10^{-7} \sqrt{\frac{(x + 100)}{src}} . \qquad (16)$$

ξ_o, as previously stated, is the value of ξ when the rubber is at zero per cent elongation. It equals 1.15×10^{-7} cm. for Compound A.

Minimum Percentage of Sulphur Required for Vulcanization—Soft Rubber

The present theory assumes that at the junction points there is a cross linking of molecular chains through the agency of sulphur atoms. Knowing the total number of junction points per cubic centimetre of compound, one may, assuming a sulphur atom at each junction point, calculate the minimum amount of sulphur necessary for vulcanization. As the values of x, s and r for Compound A will be used, the result of the calculation will be the minimum quantity of sulphur of vulcanization necessary to give an elongation at infinite stress of 850% in a typical pure gum stock. The result may also be interpreted as representing the quantity of sulphur, out of the total of 2.79% actually incorporated into Compound A, serving to form the junction points in this compound.

Now, the total number of junction points in 1 cc. is $2n^3$, and from Equation (14),

$$2n^3 = 2.63 \times 10^{18} \left(\frac{src}{x + 100} \right)^{\frac{3}{2}} = 6.98 \times 10^{19} . \qquad (17)$$

The weight of a sulphur atom is

$$\frac{32.06 \times 1.66 \times 10^{-24}}{1.008} = 5.28 \times 10^{-23} \text{ gm.}$$

The percentage of junction point sulphur, calculated on the crude rubber in the mixing, in 1 cc., or 0.955 gm. of compound, is

$$\frac{5.28 \times 10^{-23} \times 6.98 \times 10^{19} \times 100}{0.93 \times 0.955} = 0.42\%.$$

The calculation of this figure as a percentage of the whole mix makes but little difference to the result, it being 0.39% in this case.

It is interesting to note that if the value of x were increased to 1100%, without changing any of the other quantities in this calculation, the percentage of sulphur would be reduced only to 0.30%.

Minimum Percentage of Sulphur Required for Vulcanization—Hard Rubber

In the calculation of the percentage of sulphur necessary to give inextensible and inelastic rubber, a pure rubber-sulphur mix is used. The following values for s, r, c and x are supplied in the equation for the total number of junction points per cubic centimetre: $s = 0.915$, the density of pure caoutchouc (11); $r = 100\%$; $c = 100\%$; $x = 0\%$, giving the result, $2n^3 = 2.30 \times 10^{21}$. The total amount of sulphur necessary to place a sulphur atom at each one of these junction points equals $2.30 \times 10^{21} \times 5.28 \times 10^{-23} = 0.12$ gm. The percentage of sulphur would be

$$\frac{0.12 \times 100}{0.915 + 0.12} = 11.6\%.$$

Calculation of Freely Swinging Arc—Soft Rubber

An interesting calculation may be made at this point; namely, the comparison of the total number of chain carbon atoms with the total number of junction points. This gives an idea of the amount of free chain in comparison with the amount of bound chain. Using the values of s, r, c and x for Compound A, the total number of junction points per cubic centimetre was calculated to be

$$6.98 \times 10^{19}. \qquad (17)$$

The total number of chain forming carbon atoms equals

$$3.57 \times 10^{18} src = 3.02 \times 10^{22}. \qquad (11)$$

Item (11) divided by item (17) gives the ratio:

432 chain carbon atoms per junction point. (18)

As each junction point accounts for a length of chain which is the equivalent of two complete arcs, the number of chain carbon atoms lying between two adjacent junction points is the half of item (18), or 216.

Calculation of Freely Swinging Arc—Hard Rubber

The calculation of the freely swinging arc depends upon the total number of junction points in hard rubber, which, as already shown, equals $2n^3 = 2.30 \times 10^{21}$, and which depends upon x, the elongation at infinite stress. When x decreases, the ratio of the total number of chain forming

carbon atoms to the total number of junction points also decreases, *i.e.*, the amount of free chain decreases, as would be expected. When x equals zero this ratio becomes

$$\frac{3.02 \times 10^{22}}{2.30 \times 10^{21}} = 13.1. \tag{19}$$

Hence, there are approximately seven chain carbon atoms between adjacent junction points when x equals zero. According to the mathematical conception these seven carbon atoms exist in a chain which is stretched taut between junction points.

Relation Between ξ and ϵ

ϵ, the percentage elongation of the specimen, is given by the equation:

$$\epsilon = \frac{100(\xi - \xi_o)}{\xi_o}.$$

Hence

$$\xi = \frac{\xi_o(\epsilon + 100)}{100}. \tag{23}$$

Calculation of λ

When ϵ equals x, the molecular chain is stretched taut between the junction points, and $\xi = \lambda$, the half length of the molecular chain. Making these substitutions in the equation for ϵ, given above,

$$x = \frac{100(\lambda - \xi_o)}{\xi_o}.$$

Hence

$$\lambda = \frac{\xi_o(x + 100)}{100}.$$

Substitution of the value of ξ_o, obtained in Equation (16), gives:

$$\lambda = 3.42 \times 10^{-9} \frac{(x + 100)^{\frac{4}{3}}}{(src)^{\frac{1}{3}}}.$$

Relation Between A and F

It will be remembered that A is the horizontal component of the tension in the single molecular chain. F, being the stress on the specimen in kilograms per square centimetre, is equal to the sum of all the horizontal components, A, in a square centimetre of cross section at right angles to the direction of stretching. Thus F, divided by the number of chains per square centimetre, as given in item (13), and converted to dynes, equals A.

$$A = \frac{1000 \times 980\, F(x + 100)}{1.20 \times 10^{12}\, src} = 8.15 \times 10^{-7}\, F\left(\frac{x + 100}{src}\right).$$

Stress-Strain Curve Equation Beginning at -100% Elongation

By substituting the values, just obtained, for A and ξ in the equation:

$$\eta = 4A\xi C, \tag{10}$$

and giving ξ_o the value shown in Equation (16), η may be expressed in terms of stress in kilograms per square centimetre and percentage elongation.

$$\eta = 1.12 \times 10^{-14}\, FC(\epsilon + 100)\left(\frac{x + 100}{src}\right)^{\frac{4}{3}} \text{ ergs.} \tag{24}$$

Giving x, s, r and c the values which they have in Compound A, the equation becomes:

$$\eta = 4.20 \times 10^{-16} FC(\epsilon + 100) \text{ ergs,} \tag{25}$$

or

$$F = \frac{\eta}{4.20 \times 10^{-16} C(\epsilon + 100)} \ . \tag{26}$$

Stress-Strain Curve Beginning at 0% Elongation

Equation (28), mentioned earlier in this paper, which gives a stress-strain curve beginning at 0% elongation, is deduced by assuming the junction points to occupy a constant volume. In this way, the relation between ϵ_l, the percentage elongation in the direction of the stress, and ϵ_c, the percentage elongation, corresponding to ϵ_l, at right angles to the stress, may be found. ϵ_c, the cross sectional elongation, is always less than 0%, and is a measure of the contraction of one of the sides of the cross section square consequent upon stretching the specimen to an elongation ϵ_l. Letting ξ_l represent the half junction point distance in the direction of the stress and ξ_c the half junction point distance at right angles to the stress,

$$(\xi_o)^3 = \xi_l(\xi_c)^2 \ .$$

It is evident from Equation (23) that

$$\xi_l = \frac{\xi_o(\epsilon_l + 100)}{100} \ ,$$

and

$$\xi_c = \frac{\xi_o(\epsilon_c + 100)}{100} \ .$$

The substitution of these values in the above equation for $(\xi_o)^3$ gives:

$$\epsilon_c = \frac{1000}{\sqrt{\epsilon_l + 100}} - 100 \ . \tag{27}$$

Since arc rotation causes all the junction points to be attracted towards one another, the average attraction, A_c, between any two adjacent junction points in a plane at right angles to the stress, will be transmitted through the structure as a kind of hydrostatic pressure and tend to force apart the junction points along the line of the stress, thus diminishing A_l, the average force of attraction between any two adjacent junction points along the line of the stress. The average force resisting deformation, therefore, is a differential force, $A_l - A_c$, which is represented in Equation (28)

$$F_r = F_l - F_c = \frac{\eta}{4.20 \times 10^{-16} C_l(\epsilon_l + 100)} - \frac{\eta}{4.20 \times 10^{-16} C_c(\epsilon_c + 100)} \ . \tag{28}$$

The meaning of the symbols has already been given. Since F_l and F_c are proportional to A_l and A_c, it has not been found necessary to incorporate the latter two symbols into this equation. ϵ_l and ϵ_c have the relation indicated in Equation (27).

Calculation of Elongation at which the Junction Points Approach within X-ray Distances

As the junction points move farther apart in the direction of stretch they approach one another at right angles to this direction. A calculation will

now be made to determine at what elongation the junction points come within the distances between chains, measured transversely to the stress, shown in the Meyer and Mark X-ray diagram, Fig. 7. These distances are:

$$\frac{12.3}{2} = 6.15\,\mathring{A}, \quad \text{and} \quad \frac{8.30}{2} = 4.15\,\mathring{A} \ .$$

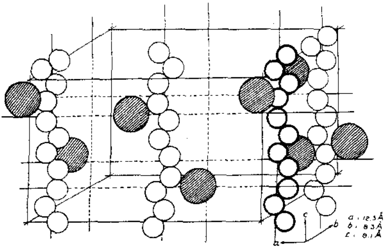

FIG. 7. *Spacing of rubber molecules according to Meyer and Mark (26), obtained from X-ray data. White circles represent carbon atoms and hatched circles represent CH_3 groups.*

The elongation at this point is found by assuming that the cell bounded by eight adjacent junction points has a constant volume, equal to $(2\xi_o)^3$. Letting y represent the distance between adjacent junction points along the line of the stress at the elongation to be calculated, $(2\xi_o)^3 = y \times 6.15 \times 10^{-8} \times 4.15 \times 10^{-8}$; therefore,

$$y = \frac{(2\xi_o)^3}{6.15 \times 4.15 \times 10^{-16}},$$

and the percentage elongation equals

$$\frac{100(y - 2\xi_o)}{2\xi_o} = \frac{100y}{2\xi_o} - 100 = \frac{100(2\xi_o)^2}{6.15 \times 4.15 \times 10^{-16}} - 100 = 1960\% \ .$$

Calculation of η

The average value of η was calculated by supplying in Equation (26) a value for F at 14.8 kgm. per sq. cm., obtained from experimental Curve A, Fig. 8, at $\epsilon = 158\%$. As F and ϵ are known, and as C can be calculated from ϵ by a method given later, η can be found, and has a value of 7.12×10^{-12} ergs. This is the elastic energy in a single arc.

Substitution of this numerical value of η in Equation (28) gives the following result:

$$F_r = F_l - F_c = 1.69 \times 10^4 \left(\frac{1}{C_l(\epsilon_l + 100)} - \frac{1}{C_c(\epsilon_c + 100)} \right) \ , \qquad (32)$$

and, above 72% elongation:

$$F = \frac{1.69 \times 10^4}{C(\epsilon + 100)} \ . \qquad (33)$$

These equations are represented graphically in Fig. 8. Curve A is the stress-strain curve drawn from an actual specimen, Curve C was obtained from Equation (32), and Curve B from Equation (33). Comments on these curves have already been made.

Calculation of the Elastic Energy per Cubic Centimetre in Rubber

When the values of x, s, r and c for Compound A are used, the single arc contains, as shown in Calculation (18), 216 chain carbon atoms, and this length of arc has, as shown above, 7.12×10^{-12} ergs of elastic energy. There

being, according to Calculation (11), 3.02×10^{22} chain carbon atoms in 1 cc. of rubber compound, the total elastic energy in 1 cc. equals:

$$\frac{7.12 \times 10^{-12} \times 3.02 \times 10^{22}}{216} = 9.94 \times 10^{8} \text{ ergs} = 24 \text{ gram-calories,}$$

or, approximately, as previously explained in Calculation (31), 17% of the total heat energy in the rubber.

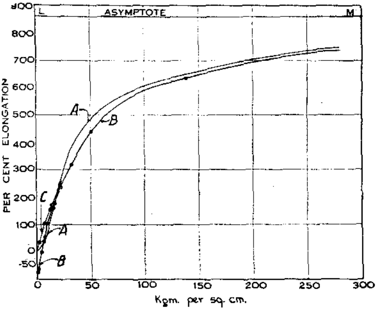

FIG. 8. *Experimental and calculated stress-strain curves. A—Experimental curve. B—Calculated curve, Equations (26) and (33). C—Calculated curve, Equations (28) and (32).*

Relation Between C and ϵ

Now

$$\epsilon = \frac{100(\xi - \xi_o)}{\xi_o}$$

and

$$x = \frac{100(\lambda - \xi_o)}{\xi_o} \quad ,$$

therefore, by algebra,

$$\frac{\epsilon + 100}{x + 100} = \frac{\xi}{\lambda} \quad .$$

But

$$\frac{\xi}{\lambda} = \frac{1 - B}{1 + B}, \quad \text{because} \quad \frac{\lambda - \xi}{\lambda + \xi} = B \quad ; \tag{9}$$

therefore,

$$\frac{\epsilon + 100}{x + 100} = \frac{1 - B}{1 + B}, \quad \text{and} \quad \epsilon = (x + 100)\left(\frac{1 - B}{1 + B}\right) - 100 \quad . \tag{34}$$

Now, when k is given, both K and E are fixed, and since both B and C Equations (9) and (10) are functions of k, K and E, as shown in Calculations (9) and (10), the values of B and C are fixed by the value of k. Again, if x is constant, which is the case for any one mix and cure, ϵ, according to Equation (34), is a function of B, so that ϵ is also fixed when k is given. Thus there is a definite value of C to correspond to each value of ϵ, this being the value for which k is the same in both of these functions.

In the tables of elliptic functions (29, 33) are given the values of K and E to correspond to values of $\sin^{-1}k$, in increments of 1°. These increments being fairly large sometimes require an interpolation to obtain intermediate values. In the case of Equation (33), where it was necessary only to find a number of points sufficiently close together to draw a curve, the procedure was to choose arbitrarily various values of k, obtain the corresponding values of K and E from the tables of the elliptic functions, then calculate ϵ and C, and, by putting these values of ϵ and C in the equation, obtain F, which gave a point on the stress-strain curve. Table II gives the results of the calculations that were made in this connection.

In the case of Equation (32) however where, incidentally, the value of k for C_l and ϵ_l is different from its value for C_c and ϵ_c, interpolation was necessary. Arbitrarily chosen values of k gave, as above, values of ϵ_l and C_l; then ϵ_c was calculated from ϵ_l by Equation (27), and the value of C_c, to correspond to ϵ_c obtained, through interpolation, by means of a chart (Fig. 9). C_l, ϵ_l, C_c and ϵ_c being known, the value of F_r could be calculated and a point on the curve obtained, F_r in this case being plotted against ϵ_l. There being, as already explained, a definite value of C for each value of ϵ, negative values of ϵ were plotted against the corresponding values of C to obtain the curve in Fig. 9. These values were taken from the ϵ_l and C_l columns in Table II.

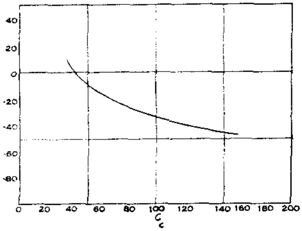

FIG. 9. *Chart relating C_c and ϵ_c.*

All the calculations are tabulated in Table II, and the stress-strain curves shown in Fig. 8 were drawn using the values given in this table. Although the second part of Equation (32) is supposed to disappear above 72% elongation, where Equation (33) is applicable, the values of F_c and F_r in Table II carry Equation (32) to 244% elongation.

TABLE II

CALCULATED VALUES OF ELONGATION AND STRESS USING THE METHOD OF ELLIPTIC FUNCTIONS

$\text{Sin}^{-1} k$	$\frac{1-B}{1+B}$	ϵ_l	C_l	ϵ_c	C_c	F_l	F_c	F_r
0°	1.000	850	0.0000	—	—	∞	—	—
10	.969	822	.0207	—	—	889	—	—
20	.885	740	.0717	—	—	281	—	—
30	.757	619	.200	—	—	118	—	—
40	.603	474	.499	—	—	59.2	—	—
50	.442	320	1.28	—	—	31.4	—	—
55	.362	244	2.17	−46	160	22.7	2.0	20.7
60	.286	172	3.91	−39	120	15.9	2.3	13.6
61	.272	158	4.44	−38	116	14.8	2.4	12.4
65	.215	104	7.73	−30	89	10.7	2.7	8.0
67	.189	79	10.5	−25	76	9.0	3.0	6.0
69	.163	55	14.7	−20	66	7.4	3.2	4.2
70	.151	43	17.6	−16	58	6.7	3.5	3.2
73	.116	10	32.1	− 5	44	4.8	4.1	0.7
74	.105	0	40.1	0	40	4.2	4.2	0.0
75	.0943	−11	50.8	—	—	3.7	—	—
76	.0841	−20	65.6	—	—	3.2	—	—
77	.0750	−29	86.2	—	—	2.8	—	—
78	.0650	−38	116	—	—	2.4	—	—
79	.0569	−46	160	—	—	2.0	—	—
80	.0479	−54	227	—	—	1.6	—	—
85	.0146	−86	3010	—	—	0.4	—	—
86	.00995	−91	6900	—	—	0.3	—	—
90	.00000	−100	∞	—	—	0.0	—	—

Conclusion

The inferences drawn from the mathematical results have been given at the beginning of the article. This order of presentation was thought to be the best, because the thread of the mathematics might be lost if the development were interrupted by lengthy comments.

To sum up, it may be stated that the mathematical consequences of a well known theory of rubber structure, the theory which assumes rotation in the long molecular chain, have been worked out, with results showing surprisingly close agreement with experiment. The correctness of the rotation theory is thus well demonstrated, in addition to which certain constants are developed which throw further light on the structure of rubber.

Acknowledgments

The author wishes to express his gratitude to Dr. G. S. Whitby, Director, Division of Chemistry, National Research Council, for his continued interest in the work. He is also indebted to C. R. Boggs, Vice-President, Simplex Wire and Cable Company, to Donald W. Kitchin of the same company, and to R. H. Gerke of the United States Rubber Company, for helpful criticisms of the manuscript.

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Additional references (see p. 491) showing the general trend of opinion toward a tri-dimensional network in vulcanized rubber are given below.

H. Staudinger, (Revue Générale du Caoutchouc, 8 : 7-75. 1931.) states that tri-dimensional reactions leave rubber insoluble. If these reactions are not extensive, as in soft vulcanized rubber, it can swell considerably, but if they are extensive, as in hard rubber, there is no swelling.

J. D. Lang, W. E. Singer and W. P. Davey, (Ind. Eng. Chem. 26 : 543-547. 1934.) consider rubber to be a tangle of spiral or zigzag molecules or molecular complexes.

THE ALKALOIDS OF FUMARIACEOUS PLANTS

IX. *DICENTRA FORMOSA*, Walp.[1]

By Richard H. F. Manske[2]

Abstract

In addition to the hitherto known alkaloids of *Dicentra formosa* (protopine, dicentrine), the present investigation has disclosed the presence of glaucine and corytuberine as well as alkaloid δ which had previously been obtained from *D. eximia*. Heyl's alkaloid of unknown identity (m.p. 142° C.) proved to be corydine. Incidentally, the author's eximine was also identified with corydine.

The object of the chemical investigations named in the above title is the elucidation of a number of problems regarding this Botanical Family*, the wider scope of which it is hoped will lead to an understanding of the biogenesis of the alkaloids. As a preliminary, however, it is essential to include in this study not only a large number of species, but to examine each as thoroughly as possible. As will appear in the sequel the re-examination of *Dicentra formosa* on this basis was warranted.

The alkaloids of this plant have been examined by Heyl (5) who demonstrated the presence of protopine and two other bases which were regarded as new. In a later examination of *Dicentra pusilla*, Asahina (1) isolated a base which he named dicentrine and which proved to be identical with one of Heyl's alkaloids. Heyl's third alkaloid (m.p. 142.5° C.) was regarded by Späth, Mosettig and Tröthandl (9) as possibly identical with the *d*-tetrahydropalmatine which these authors had found in *Corydalis tuberosa*. Support was given to this supposition by the fact that both bases exhibited the phenomenon of triboluminescence.

It seemed highly improbable, however, that but three bases should be elaborated by this plant when other species belonging to the same family are usually more liberal as to variety. In view of the development of a more adequate procedure for dealing with such refractory mixtures of alkaloids (7), a re-investigation was undertaken, and it was hoped that the exact identification of Heyl's alkaloid would be possible.

The results of the present investigation fully confirm those of Heyl. Protopine and dicentrine were readily isolated, and in a state of purity. The alkaloid melting at 142° C.† proved to be phenolic, and careful comparison disclosed its identity with the alkaloid previously isolated from *D. eximia* (8) and named eximine. Both products furthermore exhibit the characteristic violet triboluminescence.

[1] *Manuscript received April 18, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.
[2] *Chemist, National Research Laboratories, Ottawa.*

**The writer's attention has been drawn to the fact that the name Order or Natural Order is now generally applied to a much more comprehensive group of superior rank than the word Family which is used to designate those groups of genera of a closer botanical association. Chemists have not generally adopted this nomenclature. In order, however, to arrive at a common usage, that of the botanists will be used subsequently, even though there may be some confusion for a time.*

†Melting points are corrected.

In the meantime, however, the chemical examination of eximine has disclosed its identity with an alkaloid obtained from *D. canadensis* (6) and which at that time was regarded as identical with corydine. This identity has been further confirmed by comparison with an authentic specimen of *d*-corydine, which melted at 140-141° C., and for which the author expresses his indebtedness to Professor Ernst Späth. The optical activity is approximately that recorded by Gadamer (3) for *d*-corydine, and methylation with diazomethane has yielded *d*-corytuberine dimethyl ether, characterized as the *l*-bitartrate. Finally, racemization, by oxidation with iodine and subsequent reduction, yielded an inactive base melting at 168° C. Gadamer records the melting point of *dl*-corydine as 165–167° C. (? corr.).

As far as the author is aware the tribolumescent property of corydine is not a matter of previous record. That it is due to the corydine rather than to an associated impurity follows almost certainly from the fact that the *dl*-base is also triboluminescent. The problem of the melting points is still somewhat obscure. On one occasion, the base from *D. canadensis*, when recrystallized from ethyl acetate, was obtained in almost colorless stout prisms meeting at 148-149° C. Subsequent efforts to obtain the base of this melting point have yielded only a product melting at 142° C. The product obtained from dry benzene by addition of petroleum ether melted with effervescence at 124–126° C. and obviously contained benzene of crystallization, which was not lost in a high vacuum over solid potassium hydroxide and paraffin, nor in an oven at 80° C. for 24 hr. The above facts point to the existence of two crystal forms for corydine, although the alternative involving solvent of crystallization is not entirely excluded. In any case, the name eximine is to be deleted from the literature. It is relevant to state here that an attempt will be made to further characterize the author's eximidine when more material becomes available.

The close morphological relation of *D. formosa* to *D. eximia* is thus paralleled by a corresponding identity of some of their alkaloidal constituents. Differences of a quantitative nature are, however, apparent and this becomes more significant when the ratios of the components rather than the absolute amounts are considered. It is therefore of interest that, while *d*-glaucine was found only in traces in *D. eximia*, the ratio to *d*-dicentrine being approximately 100 : 1300, its present isolation from *D. formosa* was in the ratio of 100 : 70 compared to dicentrine. Obviously the process of methylenation is more readily accomplished by *D. eximia*.

A fifth alkaloid was obtained in small amounts. Its phenolic nature and solubility in a variety of solvents, as well as its melting point and color reactions, point to its identity with corytuberine. This appears to be its first isolation from a plant other than *Corydalis tuberosa*.

The investigation of *D. eximia* yielded an alkaloid which was referred to as alkaloid δ. Sufficient of the hydrochloride for analysis has become available and the data calculated for the base are in agreement with the formula $C_{21}H_{25}O_6N$. All attempts to obtain the free base in a crystalline condition

have failed. From *D. formosa* a base was isolated as hydrochloride, the properties of which were in complete agreement with those of alkaloid δ hydrochloride, and for the present the two may be regarded as identical.

Iso-corydine could not be found, although the presence of other bases in extremely minute amounts is suspected.

Finally it was of interest to determine the influence of environment upon the nature of the alkaloid constituents of this plant and for this purpose a small amount of the white flowered variety collected in the State of Oregon, U.S.A., was available. Although the total content of alkaloids was somewhat greater than in the pink flowered variety of British Columbia origin, all the bases found in the typical variety were present and the ratio of glaucine to dicentrine was approximately the same. This observation may be of some interest in that it offers a possible method of confirming botanical classification as applied to species and varieties of species. In this connection it may be noted that an exhaustive comparison of the alkaloids of *D. cucullaria* from two sources, namely, the province of Quebec and Virginia, has failed to disclose any differences other than quantitative, although those from the latter locality are uniformly more robust.

The following experiments were carried out with 11 kilos of dried whole plant material. The results of a smaller scale working with the roots only were not sufficiently different to merit separate description. The total yield of purified alkaloids was 0.40%.

Experimental

In order to obviate repetition the various designations here used have the same significance as in a communication dealing with *Adlumia fungosa* (7). For further simplification and in the interests of clarity the following outline is included:

Base hydrochlorides extracted from aqueous solution by means of chloroform,—

BC—Non-phenolic bases,—Dicentrine, Glaucine.
EC—Phenolic bases extracted from alkaline solution by means of ether,—Corydine.
BCE—Phenolic bases precipitated by carbon dioxide,—Corytuberine.

Base hydrochlorides not extracted from aqueous solution by means of chloroform,—

BS—Non-phenolic bases,—Protopine.
ES—Phenolic bases extracted from alkaline solution by means of ether,—?
BSE—Phenolic bases precipitated by carbon dioxide,—Alkaloid—δ.

Isolation of Dicentrine

The very slight solubility of dicentrine hydrochloride greatly facilitates its isolation from mixtures, and the procedure used for this purpose in the case of *D. eximia* (8) was closely followed. The base mixture (BC), which contained most of the dicentrine and all of the glaucine, was redissolved in dilute hydrochloric acid and the hydrochloride of the former allowed to crystallize as completely as possible. It was observed that a layer of ether on the surface

of the acid solution somewhat hastened this process. The dicentrine (m.p. 169° C.) regenerated from the hydrochloride and the methine prepared therefrom proved to be identical with the corresponding substances previously described (8). Yield of dicentrine: 0.10%.

Isolation of Glaucine

The aqueous mother liquor from which the dicentrine hydrochloride had been obtained was basified with excess potassium hydroxide and the liberated base collected on a glass rod, washed with water, and dried. This was suspended in a large volume of dry ether and disintegrated with a glass rod. The insoluble dark brown powdery mass was filtered off and washed with ether. The combined extract and washings were evaporated to a thin syrup.

This failed to yield any crystalline material during the course of several days. *A priori*, however, it was highly probable that this fraction should contain glaucine, and seeding with a crystal of this base from *D. eximia* induced immediate crystallization. The base was filtered off (m.p. 117–119° C.) and washed cautiously with ether; yield, 0.15%. After one recrystallization from boiling ether it was obtained in brilliant colorless prisms, which alone or admixed with an authentic specimen melted at 120° C. It may be pointed out that glaucine is generally described as being pale yellow, although the impurity responsible for the color does not lower the melting point appreciably. The methine, prepared in the usual way, melted at 75° C. (2, 10).

Isolation of Corydine

The fraction (EC) was dissolved in a small volume of hot dilute hydrochloric acid and the filtered solution (charcoal) allowed to cool. The sparingly soluble hydrochloride which then crystallized was filtered off, redissolved in hot water and the rapidly cooled solution basified with excess ammonia. The precipitated base was extracted with ether and the washed solution rapidly evaporated. Crystallization of the corydine began before all the ether had been removed. After cooling, the base was filtered off and washed with ether. It then melted at 117° C. either alone or admixed with specimens similarly prepared from *D. canadensis* or *D. eximia* (eximine). A specimen recrystallized from purified ethyl acetate melted at 142° C. and when this was admixed with a specimen of authentic corydine from *Corydalis cava* there was no depression of the melting point. Yield, 0.03%.

Another lot was recrystallized by evaporating a clear benzene solution to a small volume and treating this cautiously with hexane. After drying in a high vacuum for 24 hr. it melted with effervescence at 126° C. and had $\left[\alpha\right]_D^{25} = +214°$ (c = 1.6 in chloroform) (3).

Still another specimen was racemized by the method of Gadamer (3) and yielded colorless prisms of the inactive base melting at 168° C. All the above specimens exhibited the phenomenon of triboluminescence with great brilliance.

The base was further characterized by O-methylation with potassium hydroxide and nitrosomethyl-urethane in methanol. The non-phenolic fraction was divided into two parts. One portion was treated with an excess of a methanolic solution of *l*-tartaric acid. The *l*-bitartrate of corydine methyl ether, melting with effervescence at 222° C. (4) crystallized almost immediately.

The second portion was neutralized with methanolic hydrogen chloride. The somewhat concentrated solution was treated with dry ether until the incipient turbidity had just disappeared on shaking. In the course of several days the hydrochloride was obtained in fine needles which were recrystallized from a small volume of methanol by the addition of acetone. Corydine methyl ether hydrochloride as thus obtained is anhydrous and sinters at 239° C. and melts with frothing at 250–252° C. Calcd. for $C_{21}H_{25}O_4N.HCl$; C, 64.45; H, 6.63; N, 3.58; OMe, 31.71; Cl, 9.07%. Found: C, 63.86; H, 6.61; N, 3.85; OMe, 30.25; Cl, 9.84%.

A careful search for iso-corydine in the mother liquors from the preparation of the corydine has failed to demonstrate its presence.

Isolation of Corytuberine

This alkaloid was obtained from two fractions, namely (BC) and (BCE), its occurrence in the former being due to occlusion in the bulky precipitate of glaucine. It was recovered from the ether insoluble portion of this (*vide supra*) by dissolving in dilute hydrochloric acid and pouring the solution into aqueous potassium hydroxide. The alkaline filtrate was partly neutralized with hydrochloric acid, then saturated with sodium bicarbonate, and finally with carbon dioxide. The gray granular precipitate was filtered off, washed first with water and then with methanol. Finally it was recrystallized from much boiling water with the aid of charcoal. Colorless brilliant fine needles were thus obtained, which melted at 240–242° C., darkening and sintering taking place at 230–235° C. The fraction (BCE) was washed with methanol and the residue recrystallized from water as above. The combined yield of corytuberine was 0.02%, but owing to the somewhat wasteful procedure employed in isolating it, this figure is undoubtedly much too low. The color reactions with concentrated nitric acid (immediate deep cherry red) and with concentrated sulphuric acid (colorless; after warming, dirty pale green) are those recorded in the literature for corytuberine. Calcd. for $C_{19}H_{21}O_4N$; N, 4.32; 2OMe + NMe, as OMe, 28.34%. Found: N, 4.28; OMe + NMe, 26.06%.

Isolation of Protopine

The isolation and identification of protopine has been repeatedly described in this series of papers so that a repetition is deemed to be superfluous. The fraction (BS) on appropriate treatment readily yielded this base in a pure condition (m.p. 211° C.). Yield, 0.10%.

The mother liquors, however, yielded a small amount of colorless resinous base which could not be induced to crystallize, and so far identification has not been possible.

Isolation of Alkaloid δ

The precipitate (BSE) and the ether extract (EES) were combined, dissolved in methanol, and the somewhat concentrated filtrate neutralized with methanolic hydrogen chloride. Much dry ether was added and the pasty precipitate washed with the same solvent. Addition of a small volume of methanol, and stirring, yielded a sparingly soluble hydrochloride which was filtered off and recrystallized from hot methanol. The colorless fine prisms thus obtained melted with decomposition at 235–237° C. either alone or admixed with a specimen of alkaloid δ hydrochloride from *D. eximia*. Yield, 0.002%. No characteristic derivative has thus far been obtained, so that the question of identity is still an open one. It is to be noted, however, that seeding a supersaturated methanolic solution of the hydrochloride from one source with a crystal from another source induces immediate crystallization, and no property has been observed which would serve to differentiate one from the other. The color reaction in concentrated sulphuric acid is somewhat striking and is reminiscent of that of glaucine. In the cold the solution is colorless, but on gentle warming it becomes greenish blue, which on further heating suddenly changes to an intense and brilliant mauve.

A specimen of the purest hydrochloride obtained from *D. eximia* was dried to constant weight and yielded the following analytical figures. C, 62.31, 61.58, 62.76, 61.80; H, 6.49, 6.56, 6.33, 6.77; N, 3.57, 3.61; OMe, 23.58, 24.09%. Although these figures do not exclude a formula with two hydrogen atoms less, the formula $C_{21}H_{25}O_5N.HCl$ for the hydrochloride is at present preferred. The calculated figures are: C, 61.84; H, 6.38; N, 3.44; 3OMe, 22.92%.

Isolation of Fumaric Acid

The total quantity of fumaric acid was quite small (0.02%) so that it was found only in the ether extract (LC). The residue from the evaporation of the solvent was recrystallized several times from hot water containing a small amount of hydrochloric acid, and the fumaric acid identified by comparison with an authentic specimen (m.p. 287° C. uncorr.).

Acknowledgment

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SKRJABINEMA OREAMNI SP. NOV., A NEMATODE PARASITE OF *OREAMNOS AMERICANUS* AND *RANGIFER* SP. IN CANADA[1]

BY W. E. SWALES[2]

Abstract

An oxyurid nematode of the genus *Skrjabinema* Wereschtschagin, 1926 is recorded from the Columbian mountain goat and mountain caribou in Canada. This parasite is the first Canadian and second North American record for the genus and is described as a new species. A key to the species of *Skrjabinema* is included.

In the autumn of 1933 while studying parasitic diseases of ruminants in Alberta, the writer was enabled to make a study of the helminth parasites of game animals in the Rocky Mountain region adjacent to Jasper National Park. During the course of this investigation, which was carried out through the kindness and co-operation of Mr. S. H. Clark, Game Commissioner of Alberta, specimens of mountain goat or American chamois (*Oreamnos americanus*) and caribou (*Rangifer* sp.) were obtained from the mountainous region surrounding the Little Berland River.

A thorough post-mortem examination was made of the viscera of a young caribou and the only parasites found to be present were numerous female oxyurids which were parasitizing the large colon and caecum.

An aged female Columbian mountain goat also obtained from this region was found upon examination to be harboring many stomach worms as well as numerous oxyurids of both sexes in the large colon and caecum. This material was preserved at the time of collection and a study was made of it following the writer's return to this Institute.

The nematodes from the large intestines were found to be members of the genus *Skrjabinema* Wereschtschagin, 1926. A study of a number of males and females revealed characteristics which appeared to justify the formation of a new species, for which the name *Skrjabinema oreamni* is proposed. The following description is based on the morphological characters of 20 adult males and 20 adult females.

Skrjabinema oreamni sp. nov.

Specific diagnosis. Skrjabinema: Subgenus, *Skrjabinema* Monnig, 1932. Characters of genus and subgenus, although a slightly different interpretation of the lip membranes is given.

The Male

The male is 3.3 to 3.7 mm. long, the average mature male being 3.6 mm. in length; the maximum width is 0.175 to 0.210 mm. in the pre-equatorial

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region and the body width in the region of the esophageal bulb is 0.121 to 0.138 mm. The head is 0.047 to 0.057 mm. in diameter. The esophagus is typically oxyurid in shape, the length, including the bulb being 0.353 to 0.390 mm. The bulb is subglobular in outline 0.105 to 0.113 mm. long by 0.097 to 0.109 mm. wide.

The mouth opening is surrounded by three small T-shaped lips which are, in turn, surrounded by the wall of the mouth capsule. The lips project inward and somewhat downward. Only four head papillae could be seen in the male. The cervical expansion of the cuticle is narrow and extends to a point a little more than half way from the head to the esophageal bulb. Lateral alae are present and are 10μ wide in the equatorial region of the body and are transversely striated; they extend from the near ring to a point 0.250 mm. from the posterior extremity.

The tail is typical of this genus, being round and blunt, and having a rounded cuticular expansion, 75 to 85μ wide, supported by four lateral processes, one pair pre-anal and the other pair post-anal, each process bearing one papilla at the tip. The two adanal processes, also present, each bear three papillae.

The ventral terminal spike or cuticular fold is 16μ and the dorsal terminal spike is 20μ in length.

The single spicule measures 77 to 85μ, the head being cleft along the median line. The gubernaculum measures 22 to 24μ in length and is closely applied to the spicule throughout its length.

The heavily cuticularized rectum measures 0.173 to 0.192 mm.

The Female

The female is 9.5 to 10.5 mm. in length; the maximum width is from 0.45 to 0.55 mm., at a point approximately 1.0 mm. caudad of the vulva, and from 0.258 to 0.329 mm. wide at the esophageal bulb.

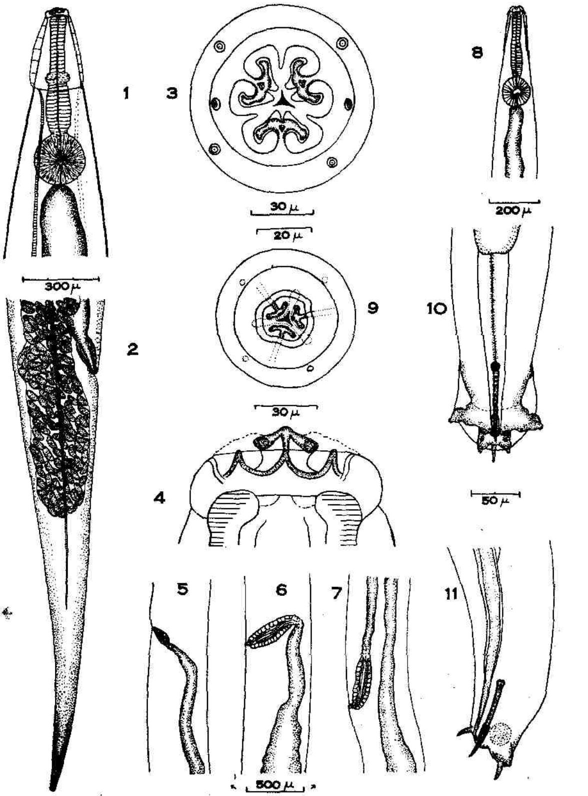
The head is 0.093 mm. in diameter. The mouth opening is surrounded by three anchor-shaped lips, each of which is encased in a cuticular membrane which projects towards the middle line in two lip-shaped structures; these structures have been previously interpreted as separate lips but dissection proves them to be directly attached to the lip membrane.

There are three small interlabia which project into the mouth opening between the lips. Three pairs of papillae are present, these being clearly visible when viewed from the anterior end.

The combined length of the esophagus and bulb is from 0.650 to 0.720 mm. The bulb is globular to subglobular, 0.188 to 0.211 mm. long by 0.196 to 0.219 mm. wide; when globular, as in the majority of specimens examined, it is 0.2036 mm. in diameter.

The nerve ring surrounds the esophagus at a point 0.240 mm. from the anterior extremity. The cervical expansion of the cuticle extends down the esophageal region for a distance of 0.275 to 0.330 mm., the expanded portion

having six coarse transverse striations. The two lateral alae are prominent, and extend from the cervical cuticular expansion to a point less than 0.500 mm. from the caudal extremity.



FIGS. 1-11. *Skrjabinema oreamni* sp. nov. *1. Anterior part of female. 2. Tail of female. 3. Anterior view of mouth structure of female. 4. Dorsal view of head of female. 5-7. Showing vulva and vagina of: 5, immature female; 6, mature female; 7, gravid female. 8. Anterior part of male. 9. Anterior view of mouth structure of male. 10. Tail end of male, ventral view. 11. Tail end of male, lateral view.*

The tail is comparatively long and tapering, being from 1.480 to 1.705 mm. in length, the average of 20 specimens being 1.516 mm. The rectum is cuticularized, 0.120 to 0.150 mm. long.

The vulva is situated at from 2.740 to 3.170 mm. from the anterior extremity, the average distance being 2.981 mm.; it is marked by a transverse slit. The vagina is wide and strong, varying greatly in position and size with the degree of gravidity in the female. In gravid females the uterus extends forward as much as 1 mm. anterad of the vulva. In the majority of specimens examined however, the uterus is entirely caudad of the vulva.

The eggs are typical of the family, one side being flat and the other strongly convex. They are 53 to 57μ in length and 30 to 35μ in width.

The main differences between *S. oreamni* and *S. ovis* are in size. Both males and females are longer and wider than those of *S. ovis*.

The female tail is always much longer.

The Genus *Skrjabinema*

Mönnig (2) lists the known species which at that time were *S. ovis* (Skrjabin, 1915) Wereschtschagin, 1926, from *Ovis aries* in Turkestan and from *Capra hircus* in Turkestan, Austria, the United States and England; *S. tarandi* Skrjabin and Mizkewitsch (5) from *Rangifer tarandus* in north Russia; *S. rupicapra* Bohm and Gebauer (1) from *Rupicapra rupicapra* in Austria; *S. africana* Mönnig (2) from *Rhaphiceros campestris* in South Africa. Mönnig did not find males of either *S. africana* or *S. alata*, but his measurements of the females appear to validate these two species. He proposed two subgenera, *Skrjabinema* and *Chilocrypta*, the former to contain *S. ovis* and *S. tarandi*, and the latter, the other three species. *S. oreamni* sp. nov. will, of course, be included in the subgenus *Skrjabinema* owing to the lips in the female not being surrounded by the wall of the mouth capsule.

Careful comparison of the description of *S. tarandi* by Skrjabin and Mizkewitsch (5) with that of *S. ovis* by Schwartz (4) and Morgan (3) does not reveal any definite difference which is constant between the two species, except in the size of the eggs. Skrjabin and Mizkewitsch show the main difference to lie in the fact that *S. tarandi* has two extra "lip parts" projecting from the middle of each lip, which are not present in *S. ovis;* however, later descriptions show these "lip parts" to be present in *S. ovis* as in all species of the genus. Similarly the differences in the head papillae and expanded cervical cuticle are not apparent when English specimens of *S. ovis* are examined. It is therefore proposed that *Skrjabinema tarandi* be regarded as a synonym of *S. ovis*, thus retaining the five other species in the genus, all of which have definite and apparently constant specific characteristics.

The known distribution of the genus is as follows:—*S. (S) ovis* (syn. *S. tarandi)* **in sheep, goats and reindeer, in Turkestan, Austria, N. Russia, U.S.A. and England.**

S. (S) oreamni in *Oreamnos americanus* and *Rangifer* sp. in Canada.

S. (C) rupicaprae in *Rupicapra rupicapra*, Austria.

S. (C) africana in *Rhaphiceros campestris*, S. Africa.

S. (C) alata in *Ovis aries*, S. Africa.

PROVISIONAL KEY TO THE SPECIES OF SKRJABINEMA

1. Lips of female not surrounded by wall of mouth capsule and project forward . . . 2
   Lips of female surrounded by wall of mouth capsule and project towards centre of mouth opening 3
2. Tail more than 1.25 mm. in length . . . . *S. oreamni*
   Tail less than 1.25 mm. in length . . . . *S. ovis*
3. Distances from head end less than 1.75 mm. and female tail more than .750 mm. long . . . . *S. alata*
   Head end—vulva 1.75 mm. or more, female tail less than .750 mm. in length . . . 4
4. Head more than 70μ broad, breadth of bulb more than 140μ (immature females only known) . . . . *S. africana*
   Head less than 70μ broad, bulb less than 140μ in breadth . . . . . . *S. rupicaprae*

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

I. THE EFFECT OF URINE ON THE EGGS AND LARVAE IN THE FECES[1]

By Ivan W. Parnell[2]

Abstract

The addition of urine, even when diluted with 25% of water, to both horse and sheep feces, proves to be lethal to the eggs and larvae of all the bursate nematodes in as short a time as 24 hr. Higher dilutions kill a large proportion of eggs and larvae in the same time, and the use of this agent is suggested as being of probable practical value in the control of these parasites.

Introduction

The two large groups into which the nematode parasites of domestic animals fall, are separated by both morphological and biological features. Economically, the more important group is that usually referred to as the Bursate nematodes (on account of the presence of an umbrella-like membrane supported by rays, called the *caudal bursa*, at the posterior end of the males). Members of this group occur in all the domestic mammals but they are specially important in horses and ruminants. In the former hosts, they are further characterized by the presence of a mouth capsule into which the mucosa of the large intestine is sucked and subjected to the action of the worm's digestive juices. This group, which in horses contains several genera and a large number of species, is generally referred to in veterinary literature as the palisade worms or *Sclerostomes*. (The name *Sclerostomum* is an obsolete synonym for the modern *Strongylus;* it is now exclusively used, following the suggestion of Stiles, for the group of worms included in the family Strongylidae found in equines.) The Sclerostomes are widespread in their distribution; practically every equine is infected with them, and undoubtedly they are the greatest single cause of disease to these animals in temperate lands.

Life Cycle

All members of this group have a similar extra-corporal life cycle. The female lays numerous thin-shelled eggs in an early state of segmentation. When passed to the exterior in the feces, these eggs develop further and give rise to a minute larva which, escaping from the shell, feeds actively, probably on bacteria, in the feces. In the course of a few days, this larva (called the first stage larva) moults, and escaping from the cast cuticle, continues to feed and grow. After a second interval, the second stage larva moults, but retains its cast cuticle as a protective sheath. This third stage larva, which is no longer capable of feeding, is the *infective* form and no further change takes place until it is swallowed by the host. It is very resistant to adverse influences, such as variation in temperature, moisture and other conditions.

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Control

From the above outline of the free life history, it is obvious that control measures must centre on some method of preventing the larva reaching the infective stage, or of preventing its ingress into the host. The latter involves methods of pasture control, segregation of horses and other considerations. The former involves some method of treating the valuable dung by drying or heating, or by the addition of chemicals. Drying to an extent which will prevent the development of the infective larva or which will kill it once it is reached, is impracticable under farm conditions. It may be employed under military conditions in dry tropical climates, by spreading the feces in a thin layer exposed to the rapid drying action of the sun. Even here however its use is limited.

The use of heat of fermentation has been ingeniously employed by Schwartz who constructed a wooden manure box into which the feces were tightly packed. The heat evolved by the fermentation in the manure destroyed all the larvae in a few days. This method, which involved the use of a special wooden box, is not generally practicable. A somewhat similar result is obtained, however, by building a compact manure heap. The internal part of the heap becomes sufficiently hot but the outer layer does not; it is necessary therefore, to turn in this outer layer every week or so and repack the pile tightly.

The addition of chemicals to the feces has been studied by a number of workers. Nöller and Schmid (8), and Nöller and Korkhaus (7) have carried out some experiments by adding kainit, ammonia salts, lime and phosphates, as well as peat and sawdust to the feces.

Parallel work on the sterilizing of human and canine feces containing eggs and larvae of the related hookworms has involved the use of ammonia salts, lime, caustic soda, salt and urine. These have given some contradictory results, but some of them are promising and suggest the possibility of adding some substance or substances to horse droppings which, while not destroying the value of the manure, would be both economical and practicable.

Experimental

Action of Horse Urine on Eggs and Larvae in Horse Feces

Experiments with Mixtures of Bedding, Urine and Water

In 1933 the writer began an investigation on the possible effect of various kinds of bedding on the eggs and larvae of a mixture of species of Sclerostomes from the horse. As a large number of species are known from this host and as a pure infection with any one form is unknown, no attempt was made to deal with a single species. Throughout these experiments however, feces from animals from the same stable was used in order to make the results comparable; in each particular series feces from the same *animal* was used.

The bedding materials used were straw, sawdust, peat-moss litter and earth. In every case, control experiments without the bedding material

were made. In the first series of experiments, the bedding was mixed wi freshly deposited feces in various proportions (see Table I) and placed i quart glass jars, with a loosely fitting lid to permit of aeration. Four pot of each mixture were prepared, two of which were *moistened* with water an two with horse urine. These cultures were incubated for about three week (15 to 27 days). To determine the number of larvae which developed, a modification of the well known Baermann technique was employed. The feces was placed in rectangular sieves, which were lowered into warm water (about 35° C.) in battery jars so that the bottom of the sieve rested about an inch from the floor of the jar, and the water just covered the top of the culture. The sieves were left for several days in position, the fluid was then placed in large filter funnels (9-in. diameter) and the larvae collected from the bottom. These two processes were repeated until all the larvae had been recovered. In *no case* were larvae found in the urine cultures. Table I shows the number of larvae found in the water cultures.

TABLE I

EFFECTS OF BEDDING ON WORM EGGS IN FECES

Weight of horse feces, gm.	Weight of bedding	Total larvae obtained	Larvae per gram of feces
60	40 gm. peat moss	2000	33
30	80 gm. peat moss	750	37
40	160 gm. soil	1300	32
40	40 gm. straw	2450	61
100	100 gm. sawdust	6200	62
100	Control	9500	95

From the figures in Table I it will be noted that the largest number of larvae developed from feces unmixed with bedding. When feces was mixed with straw and sawdust about two-thirds of this number developed, and with peat, moss and soil, less than half. While bedding seemed to have some effect on the development of sclerostome larvae, it is obvious that urine had a much more pronounced lethal action. Accordingly further experiments were undertaken to obtain more information on its action.

Further Experiments with Urine, using Modified Technique

The Baermann technique used in these preliminary experiments was clumsy and consumed considerable time; accordingly a more convenient technique was evolved. Material to be tested was enclosed in bags of cheese cloth and suspended directly in the large filter funnels. The samples were repeatedly examined and larvae were counted until only a negligible number remained. The totals referred to below accordingly represent the sum of these various examinations. In some cases, seven or eight examinations were necessary before all the larvae were recovered. Early in the experiments, in order to test whether urine suspensions, because of their greater specific gravity, had prevented larvae from falling to the foot of the funnel, the fluid from the funnels was filtered through filter paper and the paper then washed into small dishes. It was found that the presence of urine had no effect on the rate of recovery of the larvae. The Baermann technique was used throughout all the following experiments.

Feces from the same horse was placed in four quart-jars as before and *covered* with urine in two of the jars and with water in the other two. Small quantities of feces were removed from each jar at the end of three to eight days, and incubated at room temperature, after the removal of the excess fluid. After 14 and 28 days respectively, the fluid was decanted from one jar of each pair and examined for the presence of larvae. The feces was then incubated for several weeks.

No larvae were obtained in either the water or the urine; no worm eggs were found in the urine, but a few were found in the water in an undeveloped condition. From the feces soaked in water (from 3 to 28 days) larvae subsequently developed, but in no case did any develop from feces soaked in urine. A parallel series of experiments using cow's urine instead of horse's, was carried out with identical results. Urine proved lethal to both eggs and larvae, while water proved merely to have a delaying action on the development of the eggs, although prolonged immersion in water appeared to kill some of the eggs.

Effect of Possible Farm Practice

These three series of experiments having shown that urine had a lethal action on the eggs and larvae of horse sclerostomes, a series of cultures were made to test, on a laboratory scale, the effect of possible farm practice, and to determine the concentration of urine and time of exposure necessary to destroy the eggs and larvae.

Fresh horse feces (50 gm.) was used for each culture and mixed with various amounts of horse urine and pure water, only sufficient fluid being used to wet the feces. The feces was examined after 28 days by the modified Baermann technique described above; the results are shown in Table II.

TABLE II

EFFECTS OF VARYING DILUTIONS OF URINE ON WORM EGGS

50 grams of feces moistened with	Larvae obtained
Undiluted horse urine	Nil
Three parts horse urine with one part water	Nil
One part horse urine with one part water	80
One part horse urine with three parts water	2000
Pure water	17600
Not moistened	59000

The Nature of the Action of the Urine

In order to determine whether the fluid had merely delayed the development of the eggs or had actually killed them, the cultures, drained of surplus moisture, were cultivated. It will be appreciated that the large quantity of water used during the Baermann process (which occupied five days) reduced the amount of urine present to a very small amount. After culturing for 12 days, the feces was again examined by the Baermann process. Feces which had been moistened with 100, 75 and 50% urine, produced no larvae. From that moistened with 25% urine, 1650 larvae were obtained and from that moistened with water alone, 2900 were obtained. The unmoistened control produced 1700 in addition to those previously obtained.

TABLE III

RESULTS OF CULTURING FECES AFTER ACTION OF VARYING DILUTIONS OF URINE

Feces moistened with	Larvae obtained
Undiluted urine	Nil
Three parts of urine plus one part water	Nil
One part of urine plus one part water	Nil
One part of urine plus three parts water	4200
Pure water	30000
Unmoistened	28000

A similar series of cultures examined after 38 days produced almost similar results (Table III).

Effect of Time

A series of experiments was undertaken to determine the length of time during which urine has to act on horse feces to prevent the development of infective larvae, and whether stale urine is as effective as fresh. Samples of feces (50 gm.) were again used in each case and the cultures *moistened* with the liquids. To facilitate subsequent handling, the feces was loosely wrapped in muslin bags. Samples were wetted with stale urine (although no free fluid was present in the jars) and after 24, 36, 48, 60, 72 and 96 hr., respectively, were squeezed as dry as possible, moistened with pure water and cultivated for about a fortnight at room temperature. They were then examined as before for the presence of larvae. In *no case* were larvae found. Samples similarly moistened with fresh urine for 36 and 60 hr. respectively were also negative, while control samples treated with water produced 5200, 800, 240 and 2200 larvae respectively after 24, 48, 72 and 96 hr. respectively.

Similar experiments with cow's urine showed that its effect was identical with that of horse urine.

Effect on Larvae at Various Stages of Development

To ascertain the stage in development at which urine is most lethal, another series of cultures was made. Fresh feces in four sets of jars, each containing 50 gm., was cultivated for one, two, three and four days respectively and then soaked for 24 hr. in horse urine; the fluid was then decanted and the feces thoroughly mixed with about 10 times its volume of water. Little, if any urine could have been left in the feces which was squeezed dry, moistened slightly with water, and cultivated for about a fortnight. The wash water was filtered and the filtrate was also similarly cultured to recover any eggs or larvae which might have otherwise been lost during washing. In none of these cases did larvae develop.

Two sets of feces were soaked in urine for 24 and 48 hr. respectively; the urine was then squeezed out and the feces was cultivated as before (after washing) for a fortnight, and examined for larvae. In the first case, 140 larvae were recovered, while in the second the culture was negative. In controls similarly treated with water instead of urine, 25,000 and 15,000 larvae respectively developed. As there was no surplus urine in these cultures, it is possible that a small area on the top escaped the action of the urine and that this accounts for the 140 larvae, rather than the fact that the action of the urine was only partly lethal.

Simultaneously with the previous experiments, feces from the same horse was cultivated in 50-gm. lots for a fortnight. One lot was then soaked in urine for 24 hr. and a second for 48 hr. and both feces and urine examined for the presence of larvae. In the case of the sample soaked for 24 hr. in urine, 15,000 larvae, and in that soaked for 48 hr., 2200 larvae, were obtained. In both cases all the larvae were dead. In the controls, treated in an identical manner, but with the substitution of pure water for urine, 65,000 and 50,600 active, living larvae respectively were obtained.

These experiments show that urine of the horse and the cow has a lethal effect on both the eggs and larvae of the horse Sclerostomes in manure, while water has merely a delaying action on the development of the eggs, although a portion of the eggs are destroyed on prolonged immersion in water. Undiluted urine as well as urine diluted with 25% of pure water kills all the eggs and larvae, but a large proportion is destroyed by as low a concentration as 25% of urine in water, in as short a time as 24 hr.

Action of Urine on Eggs and Larvae in Sheep Feces

A related group of worms is one of the commonest sources of loss among sheep, and the opportunity was taken of the presence of heavily infested sheep at the Institute to test the effect of urine on the eggs and larvae present in their feces. Samples cultivated for 30 days after being treated with concentrated urine or urine diluted with 25% of water were free of larvae, but a large number of larvae developed when solutions of lower concentrations were used. It was interesting to note that more larvae developed from unmoistened feces than from feces moistened with pure water. A second series of experiments, identically treated but cultured for 37 instead of 30 days, yielded no larvae from samples treated with concentrated, 75, 50 or 25% urine. The controls again gave a heavy culture.

To ascertain whether urine had been lethal to eggs in sheep feces or early stage larvae and had not merely delayed development, the second series of sheep feces was recultivated for 12 days, it being again assumed that since the wash water was many times the volume of the highest concentration of urine, at least some larvae would develop if they had not been killed by the urine. A number of larvae developed only from the 25% dilution of urine. As this number was only about $\frac{1}{15}$ of that obtained in the controls, it may be assumed that even the weakest dilution was partly lethal to the eggs and wholly so to the larvae.

Conclusion

Urine accordingly appears to be a possible agent which could be used in the practical control of bursate nematodes in horses and in stabled sheep. Not only is it available simultaneously with the feces, but it costs nothing beyond the cost of collection and it not only does not lessen the manurial value of the dung but actually increases it.

Experiments are at present in progress at the Institute of Parasitology to ascertain the most economical and practical method of using urine under actual farm conditions as a method of controlling what is now the most serious and widespread cause of disease in horses, and which is found in all civilized countries.

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COLOR CHANGES IN SNOWSHOE HARES[1]

By S. Hadwen[2]

Abstract

Observations on the color changes in *Lepus americanus virginianus* in Ontario are recorded. The production of pigment may be influenced by the fact that the hares can have more than one litter per season. The skin is thicker in the unprime condition than in the prime. From a study of melanin formation in the hair roots it was found that there are produced in succession several kinds of pigments which form the color pattern in the full grown hair. The structure, percentages, and types of hair are discussed. Kempy fibres are recorded. A different type of hair on the soles of the feet is described. This hair apparently grows independently of the hair on other parts of the body. Reasons are given for the erectness of winter fur as opposed to flat lying young summer fur. Hares were clipped on one side of the body. In two cases the regrowth of fur produced bands of color and then whitened above; the hairs were identified by the cut ends. Dyeing experiments showed that when brown hair whitened it lost part of its own natural pigment and the dye as well. In two cases where the tips of the hairs had been cut, the dye ran inside and permanently marked them.

Introduction

The nature of the color changes that take place in the fur of northern hares has long been an unsolved problem. The experiments described here were planned to show what alterations actually occurred in the skin and hair of living animals. The two main objects of the investigation were (i) to prove that the blanching of the fur of snowshoe hares is caused by a process of depigmentation and masking of pigment in the hairs, and (ii) to confirm the writer's previous observations (10). The observations were made in Ontario on *Lepus americanus virginianus*, which lives in the northern part of the province. A previous paper (10) dealt with the Manitoba snowshoe hare, which differs a little from the Ontario form, but has the same habit of turning white in winter; therefore, in relation to the pigmentary changes, the two subspecies may be regarded as similar. Two methods were tried, *i.e.*, clipping the fur, and dyeing it before it turned white.

That the whitened fur still contained pigment was proved by microscopic examination. The disappearance of melanin had to be shown by experiment. In general it may be stated that the observations confirm the results obtained by the writer (10) in 1929. All photographs in this article are from untouched negatives.

Experimental Animals

General Remarks

The experiments began in November 1932 and continued up to 1934. About 50 hares were either captured alive or shot, and 20 were caged for varying periods. Five hares were alive at the conclusion of the work. Eight captured hares were caged from May 1933 to the close of the year.

[1] *Manuscript received March 19, 1934.*
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Table I gives a general idea of the coloring of the animals at different periods. The first change noted was the whitening of the ears and feet, which began as early as September 6. The more striking changes which start later, such as blanching of the face, usually take about 30 days (average of 10 animals) but they may take longer—up to 60 days or more. The first of the caged hares to become white did so on November 1, and the last about January 10.

Starting in April when the white portion of the fur was shed the hares turned brown, except for a little white which remained on the face, ears and feet. In May and June the backs of the ears became devoid of hair. On June 23, out of eight newly caught hares, four had white skins and four dark. The white-skinned hares had the normal summer coat and their ears were furred. The dark-skinned animals had poorly furred ears and the darkness of their skins showed that the hair had not finished growing. From July onward there was no noticeable moult in any of the caged hares, except in one instance, No. 23. This animal had a short woolly coat up to September 26.

Among the hares which were shot during the summer several moulting specimens were secured. It will be noticed in Table I that a thin white prime skin was recorded as early as May 12, but most of the white skins were collected from July onward. As the prime condition of the skin means that the hair has ceased growing and must be replaced later by new hair, these observations should be continued in order to determine exactly how long it takes for the animals to grow a new coat of fur, and whether this may be accomplished more than once during the summer. This point is discussed later in connection with the experimental clipping. Two hares, No. 34 and one which is still alive, did not whiten completely. No. 34 had a small patch of brown on the forehead on January 10. One of the captured hares (No. 32) died of pneumonia. This animal had an abnormal coat. There were a large number of short degenerating white hairs near the skin. These hairs had whitened before they had completed their growth and it is possible that they ceased growing when the hare became ill. This is the only abnormal coat found in the series.

The caged hares were kept in separate pens and consequently had no chance to mate. The animals were housed in a steam-heated room and at no time were subjected to cold. A caged hare began to shed its white coat on February 20, another on March 9. Large numbers of guard and pile hairs with roots attached were found on the bottom of the cages.

Number of Litters

As the bearing of young may have an effect on the coat, it must be taken into consideration, and therefore the breeding habits of the hares were noted whenever possible. It would appear that Ontario hares may have one or more litters per year. This is based on the fact that pregnant "does" were shot at different dates during the spring and summer. On July 25 at Geneva Lake a doe that was milking was shot and found to have four small fetuses

TABLE I

CHANGES IN SKIN AND HAIR OF RABBITS

Skin No.	Date received	Received from	Appearance of hair	Appearance of skin	Microscopic appearance
	1933				
1	Jan. 15	Cartier, Ont.	White	White and thin	Root ends frayed
2	Jan. 15	Cartier, Ont.	White	White and thin	Root ends frayed
3	April 12	Cartier, Ont.	White, soles of feet blue	Slight spotting	Root ends frayed. New pigmented roots
4	April 17	Cartier, Ont.	White, soles of feet blue	Spotted brown and black with new growth below the hair	Root ends frayed. New roots red and black
5	April 23	Cartier, Ont.	Mostly brown	Very dark and blotchy	Pigmented roots
6	April 25	Cartier, Ont.	Ears white and brown	White with slight spotting	Frayed roots and pigmented roots
7	April 26	Kirkfield, Ont.	Almost brown on back. A few patches of white. Soles of feet blue	Darkish line along skin of back	New roots red and black
8	April 26	Kirkfield, Ont.	White. Ears white	Very black	Pigmented roots
9	April 27	Cartier, Ont.	Brown and white	Dark brown and black	Pigmented roots
10	April 29	Cartier, Ont.	Mostly brown. Ears brownish, feet blue	Spotted	Frayed roots and pigmented roots
11	April 29	Cartier, Ont.	White. Ears white. Feet blue	Black and brown	Pigmented roots
12	May 1	Cartier, Ont.	Brown and white. Ears white	Blotchy	Pigmented roots
13	May 2	Cartier, Ont.	Brown and white. Feet blue	Very dark	New roots pigmented
14	May 12	Cartier, Ont.	Hair brown. Back of ears bare; soles of feet blue	Nearly white and few spots present	Frayed roots and pigmented roots
15	July 25	Geneva Lake, Ont.	Very dark; back of ears bare	Thin with few black spots	Skin white; no black roots
16	July 25	Geneva Lake, Ont.	Short. Ears bare, feet brown, soles dark	Mottled black	Hair roots degenerated; only small ones left
17	July 25	Geneva Lake, Ont.	Fur dark and new looking. Ears naked at back. Feet brown with few white hairs. Soles dark colored	Whitish; few black spots	New roots growing black and brown
18	July 27	Geneva Lake, Ont.	Brown. Ears partly bare	Thin and white	Roots frayed
19	Aug. 2	Geneva Lake, Ont.	Fur loose on back. Ears naked; feet brown; soles dark	Almost black	Large roots becoming pigmented; small roots black; no new brown roots. A few large roots growing on side
20	Aug. 2	Geneva Lake, Ont.	Hair shedding. Backs of ears naked. Feet brown; soles dark. Still a few white hairs attached (winter?)	Whitish except for few spots	Return of pigment brown and black in spots
21	Sept. 17	Cartier, Ont.	Ears and feet brown. Soles of feet dark. Hair brown and erect	Bluish	Last traces of pigment in roots. Outlines of roots in melanin (?)
22	Sept. 25	Cartier, Ont.	Erect; ears whitish; feet brownish; soles whitish	Thin; very slight spotting	A few fine hair roots with degenerative pigment
23	Sept. 26 (caged)	Cartier, Ont.	Scanty yellow hair matted and old. Ears and feet whitish. Soles white. Above yellow line regular brown fur	Dark except yellow line along back, where it was thick	On line in centre of back, new brown and black hair roots; on dark parts of skin only black roots; yellow area more vascular than black

TABLE I—*Concluded*

CHANGES IN SKIN AND HAIR OF RABBITS

Skin No.	Date received	Received from	Appearance of hair	Appearance of skin	Microscopic appearance
	1933				
24	Oct. 6	Cartier, Ont.	Erect and brown. Whitish feet and ears; soles whitish	Thin and whitish	No pigment in hair roots; ends frayed
25	Oct. 10	Cartier, Ont.	Brown and erect. White ears and feet	Thin and whitish	No pigment in roots; ends frayed
26	Oct. 18	Cartier, Ont.	Nearly white	Thin and nearly white	All roots frayed at ends
27	Oct. 18 (caged)	Cartier, Ont.	White and erect. A few black spots on flank. No growth on clipped side on feet and ear	Thin and white	A few pigmented hair roots on clipped side; ends frayed
28	Oct. 18 (caged)	Cartier, Ont.	Erect. Soles of feet whitish	Whitish except line along back	A few small roots with degenerated pigment on line along back
29	Oct. 19 (caged)	Cartier, Ont.	Unclipped side normal brown. Brown and fawn on clipped side. Feet brownish; soles white. Clipped ear and feet no growth	Yellow line on back. Rest of skin blackish	Only small hairs with black pigment. Large roots degenerated
30	Oct. 19 (caged)	Cartier, Ont.	Erect and nearly white. Feet whitish; soles white	Thin and whitish. Black along back of head	Only small roots with degenerated pigment
31	Oct. 19 (caged)	Cartier, Ont.	Dark. Fawn on clipped part	Dark and thick	Hair roots degenerating
32	Oct. 23–Nov. 1	Cartier, Ont.	Brown	Very thin and white	A very few pigmented small hair roots; other roots frayed at ends
	1934				
33	Jan. 9	Cartier, Ont.	White	Very thin and white	All roots frayed at ends
34	Jan. 10	Cartier, Ont.	White	Thin and white	All roots frayed at ends
35	Jan. 15	Cartier, Ont.	White	Thin and white	Roots frayed at ends. A few very small black spots with active roots
36	Jan. 18	Cartier, Ont.	White	Thin and white	Roots frayed at ends. A few very small black spots with active roots

about two weeks old. Additional data obtained concerning litters and fetuses are as follows. On April 26, two fetuses were found in a doe, and on May 4, six were found in another. On May 19, two young were born to doe No. 7; on May 21, one to doe No. 5; and on May 22, three to doe No. 6. On July 23, four fetuses were found in a doe; and on July 25, three, two, and four fetuses were collected in different "does".

Mr. Stewart Criddle of Manitoba informs the writer that snowshoe hares in that province have two, or possibly three, litters per season.

Grange (7) states, "One captive female produced two litters during the same summer. Whether or not this occurs in a state of nature we do not know."

The Skin

Pigmentation

The pigmentation of the skin, with the exception of the exposed parts such as the eyelids, is due to the pigmented hair roots which show through the colorless epidermis. In the spring, black patches appear in the region of the tail head. These spread out over the body, extending rather irregularly downward over the flanks. In autumn, the reverse process occurs, that of whitening, starting from the feet and extending upwards. The last portion to lose its pigment is a line over the backbone. Primeness of the skin occurs both in summer and winter. But there is a difference in the primeness in these seasons; in winter when the hair is white the skin is always prime, whereas in summer when the fur is brown the skin may be prime or unprime according to the growing condition of the hair.

Measurements

The skin of hares varies in thickness at different seasons, and these variations are intimately connected with the growth or death of the hair roots. The period of growth is accompanied by increased vascularity, glandular activity and thickening.

Through the co-operation of Mr. Henry of this Foundation, 17 skins were measured, and though this is perhaps too small a number to serve as a basis for a definite statement, nevertheless there was a distinct difference in thickness between prime and unprime skins.

Primeness in the Skin of Other Animals

Blanching of the skin occurs in animals such as the muskrat, which, unlike snowshoe hares, do not whiten. The blanching stops just above the hair roots. This difference has been interpreted as showing that the process in hares is not a destructive depigmentation. This, however, does not appear to be correct as once a hair root has become frayed at the end it is dead and cannot return to an active state of pigment formation.

On examining the ears of hares which had turned white, it was found by the writer that on the edges and also at the tip where the hair remains colored, the roots whiten and become frayed at the ends, just as they do in the muskrat, and though the areas in which this occurs are small they show the same type of depigmentation of the roots without extension up the hairs.

The Hair Roots and Pigment Formation

Fig. 5, Plate I, shows the egg-shaped hair bulb with a clear space at the end. Surrounding the hair as it emerges from the bulb is a well marked sheath which continues up above the root. It must vary considerably in diameter owing to the uneven width of the hairs. Blood vessels surround the bulb and may be seen in Fig. 6, Plate I. No arrector pili muscles have been demonstrated though they have been mentioned by other writers.

The formation of melanin is entirely confined to the root. When the hair roots begin to grow, melanoblasts collect in small groups, and if they are

examined in a clear area of the skin no other cells appear to accompany them (Plate I, Fig. 8). As the cells reproduce, the tip of the new hair is formed. A noticeable feature in the new hair tips is presented by the spiral twists which remain at the ends of the fully grown hairs. The twists occur in clear transparent areas where there is seemingly no resistance offered to the advancing tip, except that due to the fibres of the skin itself. It has been suggested that spirals are due to the moulding effect of the epidermal cells, but this is evidently not the case, the spirals being formed early in the growth of the roots (Fig. 1). The root then becomes more clearly defined though the sheath is not formed until the hair has grown longer. At this stage there is a sudden alteration in the character of the pigment in the roots from black to a fine reddish brown. After a further period of growth the brown melanin changes again to slightly coarser dark brown pigment. The last stage of pigment production in the root is the longest and represents a growth of hair of 1–1½ cm. and the pigment is made up of coarse oval-shaped grains. All the stages mentioned may be seen in the figures in Plate I. The hair roots in similar stages have a uniform appearance, but pigment formation does not proceed evenly in all the roots at the same time, as they are in different stages of growth, although the precursors of melanin formation must be present in all parts of the skin (Plate I).

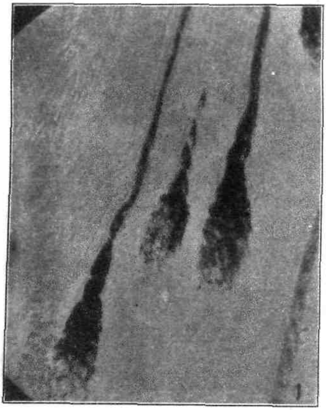


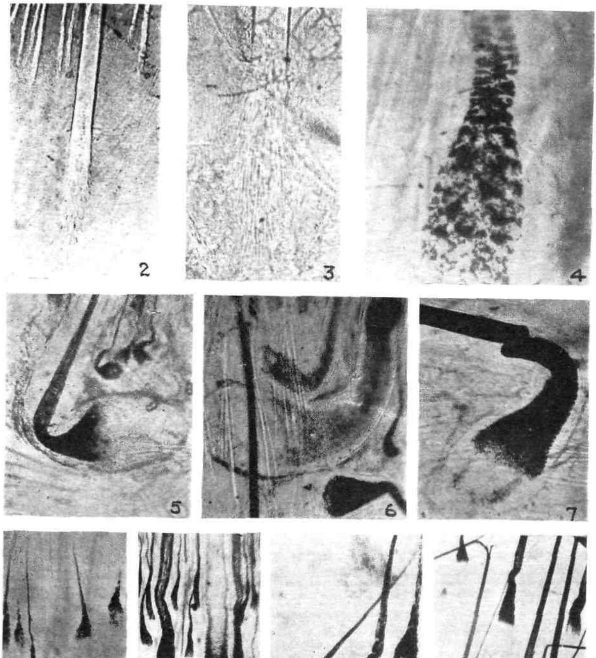
FIG. 1. *The young hair root in the centre shows the individual cells pointing in different directions, forming a spiral. In the roots on either side the process has gone further.*

The Fur

General Description

In summer the fur is banded as follows: from the skin outward the colors are blue-black, fulvous, dark brown, light yellow and black. In winter it is bluish-black, fulvous, dark brown and white. The summer pelage corresponds with the colors produced in the hair roots. The same general banding may be seen in the fur of other animals, such as deer and squirrels.

The principal difference between the summer and the winter coat is confined to the guard hairs and the pile, which whiten after the skin becomes prime. These winter hairs not only whiten at the tips but, in the case of the guard hairs, they have a whitish appearance right down to the skin. The word whitish is used because these hairs are actually of a bluish color



FIGS. 2-3. *Straight roots in prime winter skin. Note broken bits of tissue above frayed roots.* FIG. 4. *Hair root losing its pigment.* FIG. 5. *Hair root showing sheath. (About 230 diam.)* FIG. 6. *Root with encircling blood vessels; producing rusty pigment. (About 230 diam.)* FIG. 7. *Root producing black pigment. Note kink above. (About 230 diam.)* FIG. 8. *New hairs. The faintly colored roots were once black but now are producing rusty pigment; later they will produce black pigment, as in Fig. 9.* FIG. 9. *Change in pigment production from red to black.* FIG. 10. *Young hairs, bulbs forming. (High power.)* FIG. 11. *Hairs bent at point of exit through the skin. (Low power.)*

when pulled out and examined over a piece of white paper. They constitute a small percentage only of the white coat (16–25%) and are not numerous enough to whiten or obliterate the colored bands below. The pile hairs which constitute 75–85% of the white fur have an attenuate base so that they become invisible in the colored bands (Fig. 28).

The undercoat of snowshoe hares does not bleach. This is the reason why the bands remain after the outer coat has changed color. In the larger hare, *L. campestris*, the undercoat whitens almost completely on most parts of the body.

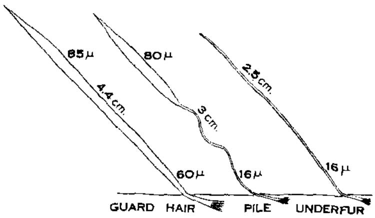


FIG. 28. *Illustrating three main types of hairs.*

It should be mentioned that there are present a certain number of hairs which are intermediate between guard hairs and pile, but not in sufficient numbers to materially alter the percentages given.

Percentages of Guard and Pile Hairs

The percentages of guard and pile hairs were estimated by the writer by mounting small locks of hair on slides and counting the number of large hairs which reached the skin end of the lock. This gives the number of guard hairs. The slide was then moved forward and the number of wide hairs counted at the free end. This gave the total number of guard and pile hairs. From these figures the percentages of each could be calculated.

Measurements of Hairs

Data obtained on the measurements of hairs are shown in Table II. The

TABLE II

MEASUREMENTS OF HAIRS

—	Summer fur	Winter fur	—	Summer fur	Winter fur
Blue-black	1.3 cm.	1.5 cm.	Width of guard hairs:		
Fulvous	0.5 cm.	0.5 cm.	Outer end	84.8 µ	85.0 µ
Dark brown	0.5 cm.	0.5 cm.	At skin	60 µ	60 µ
Light yellow	0.5 cm.	No longer present	Outer coat	25%	16%
Black	1.0 cm.	Only slight amount at tip	Width of pile hairs:		
			Outer end	78.0 µ	80.0 µ
White	—	2.5 cm.	At skin	16.0 µ	16.0 µ
Length of guard hairs	3.8 cm.	4.4 cm.	Outer coat	75%	84%
Length of pile hairs	3.0 cm.*	3–3.5 cm.*	Under-fur	2.5 cm.	2.5 cm.

NOTE:—*See Figs. 12, 13, 14 and 15.* **Approximately.*

total length of the summer fur, taken shortly before the change to white, i 3.8 cm. The winter coat is 0.6 cm. longer. There is not as much differenc in length, as many believe, between the full grown erect summer hair an the winter fur, as the latter is white, giving the impression of greater length

The measurements show the width of the hairs in the pigmented zones. The width of the hairs affects the coloration of the bands both in winter and summer, and the percentage of the different types of hairs also contributes to the banded coloration of the fur. To avoid confusion an explanation as to the exact meaning of white, as applied to hair, is necessary. Strictly defined, a white hair is one which contains no pigment. But the bleached or whitened hair of the snowshoe hare contains a little pigment at the tip and a considerable amount at the base, which means that it is not pure white like albino hair; the white effect, then, is due to reflecting surfaces which obscure the underlying pigment.

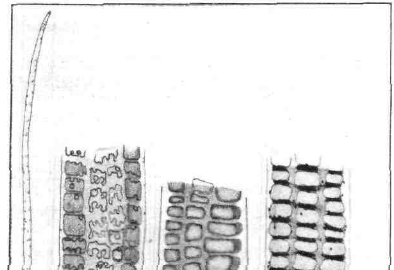


FIG. 12. *Tip of guard hair showing pigment and spiral turns.* FIG. 13. *Wide portion of hair with air bubbles and degenerate cells. This was formerly the yellow zone.* FIG. 14. *Zone in which pigment has disappeared. The cells are widely separated.* FIG. 15. *Blue-black zone near skin.* (*Drawn by A. M. Fallis.*)

Summer Fur (Microscopic Appearance)

The guard hairs in the summer fur are stiff supporting hairs, as the name indicates. From the tip, which is coal black and shiny (9 μ in width), down, the hair widens rapidly and the black pigment fades out as it reaches the yellow zone where the hair is widest (see Figs. 12–15, which are drawn to scale). Here the cuticular strip shows as a shiny wide transparent zone surrounding the hair. The shiny cuticle has the same effect as shellac over patent leather, enhancing the yellow color. It is in this yellow area that air bubbles are numerous both inside and outside the hair cells (Plate III, Fig. 23). Farther along the hair, the cuticular strip narrows down, owing partly to the presence of small dark

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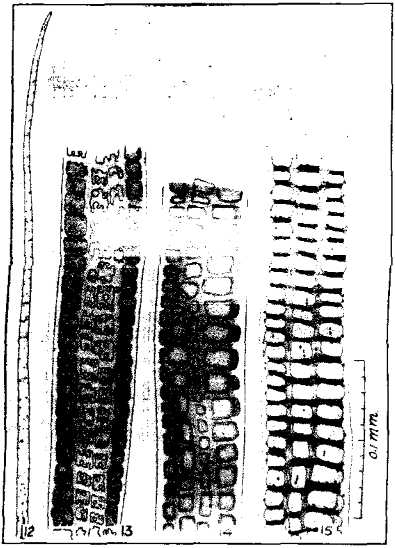

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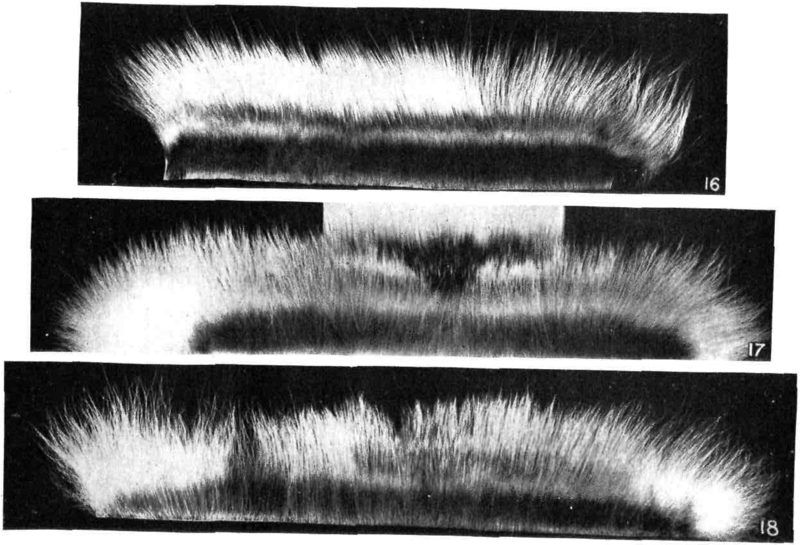


FIG. 16. *Prime winter fur.* FIG. 17. *Changing fur. The dark brown portion at the centre is turning white and is isolated from the rest of the coat. This furnishes evidence of the change of color in existing fur.* FIG. 18. *Section through the fur of Hare 30. Clipped and dyed with Aniline blue. The color bands are irregular on left side. Note cut ends of hair. Re-grown hair not as long as on unclipped side.*

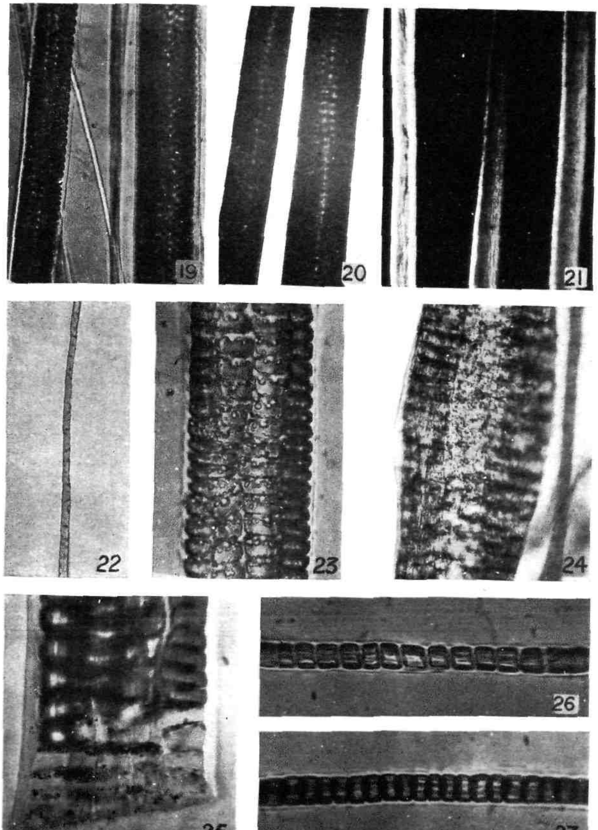


FIG. 19. *White albino hair; domestic rabbit. (About 160 diam.)* FIG. 20. *Black hair; domestic rabbit. (About 160 diam.)* FIG. 21. *Black and white hairs; snowshoe hare.* FIG. 22. *Tip of whitened hair still containing pigment. (About 360 diam.)* FIG. 23. *Air bubbles inside and out of hair cells. (About 360 diam.)* FIG. 24. *Finer type of pigment which disappears. (About 360 diam.)* FIG. 25. *Whitened guard hair with pigment grains. (About 360 diam.)* FIG. 26. *Base of pile hair near the skin.* FIG. 27. *This is the same hair as in* FIG. 26 *but closer to the skin. Note spaces between cells tightly packed with melanin.*

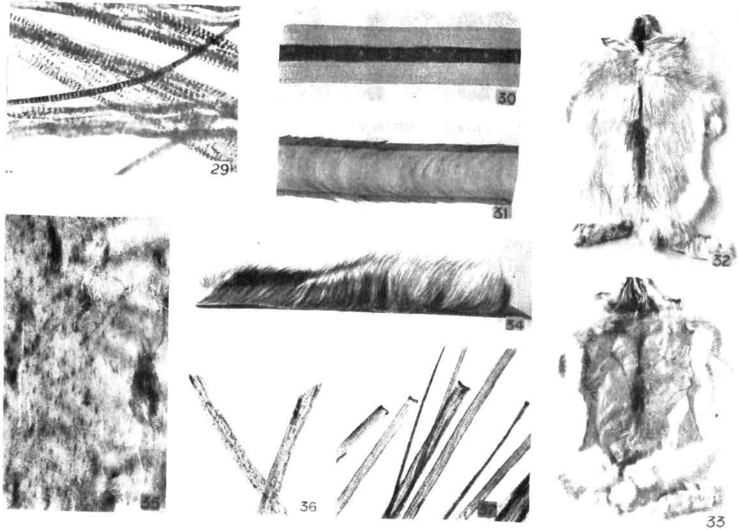


FIG. 29. *Kempy fur. Twisted distorted fibres.* FIG. 30. *Hair from sole of foot. Note wide cortical strip.* FIG. 31. *Hair from sole of foot. Outer portion. Note fringe which is produced by wear.* FIG. 32. *Note dark line in fur—the last part to whiten.* FIG. 33. *Underside of skin seen in Fig. 32. This shows that the pigment in the hair roots coincides with hair color.* FIG. 34 *Skin cut lengthwise along back, showing erectness of light fur compared with that of the dark fur.* FIG. 35. *Unprime spots in skin.* FIG. 36. *Hairs from hare No. 30, tipped with Aniline blue.* FIG. 37. *Red tipped dyed hairs from hare No. 34.*

brown grains of melanin which take up space and give the hair a dark coloration. Below this area the hairs often show irregularities in width but broaden out again, and from here down to the skin become heavily banded with large oval-shaped grains of melanin. The grains of pigment lie close to the hair cells producing the blue-black coloration (Fig. 25, Plate III).

In 1929 the writer (10) showed that the attenuate base of the pile hairs breaks off readily when the hair is pulled, and an experimental hare was plucked in the white state. In examining moulted hairs on the bottom of the cages in 1934, he found that, when naturally shed, both pile and guard hairs have their roots attached. It would therefore appear that many of the hairs are shed intact and that it is not necessary for them to break off above the root at moulting time.

Winter Color of Hairs

The winter and summer hairs differ but little, except as regards color and length. The guard hairs are about a centimetre longer in winter and the other types of hairs lengthened in proportion. The whitening of the guard and pile hairs is due to two principal causes, the first being a degeneration and shrinking of the fibres and the second a disappearance of melanin.

Both guard hairs and pile become twisted and flattened. The centre collapses and the sides of the hairs become rounded. The cuticle becomes very shiny and glassy, and has a mirror-like effect. The cells in the hairs shrink and degenerate so that light passes between them. This effect and that of the shiny cortical strip produce the effect of whiteness. The finer types of pigment, the yellow and the fine dark brown, are the ones that disappear. At the tip of the hair, following the spiral twists, a small amount of black pigment remains. This pigment and the coarse black pigment at the base appear to be much more resistant to change than the finer types of melanin.

It has been stated that the production of pigment in all the roots does not occur simultaneously, and the same thing applies to the whitening hairs, which do not all become white at once but only in certain areas at a time. For instance, the guard hairs whiten a little in advance of the pile and, being longer, are plainly visible over the dark under-fur.*

Air Bubbles in the Hair

Air bubbles in the hairs are mentioned by a number of writers. Welsh (16), Poulton (13) and Verne (15) believe that the white color of the hair is due to air bubbles. The writer mentioned air bubbles in 1929, and at that time he thought that they contributed to the white coloration. Since then he has found that bubbles occur in colored hairs; therefore it cannot be said that they cause the white coloration.

**The disappearance of melanin in the hairs may be very clearly seen in the long vibrissae on the face. These hairs have a horsehair type of conformation. White patches appear, often, about the middle of the bristle. The cortical part becomes white and transparent and the fine granular pigment fades away. In the snowshoe hares the vibrissae whiten about the same time as the fur.*

Nevertheless, through their reflecting surfaces in conjunction with the hyaline cortical strip, bubbles may enhance the white effect.

Kempy Fibres

Kempy fibres are frequently observed in snowshoe hares. These fibres differ from the hairs in the whitened coat, inasmuch as the degeneration has gone much further. The outlines are distorted and the hairs consist of little else than the shell of the former hair (Plate IV, Fig. 29).

In the regrowth of fur which followed the clipping of three of the experimental hares, Nos. 4, 5 and 7, there were many of these fibres, not only of guard hairs and pile but of the underfur likewise.

It should be mentioned that these kempy hairs grew up from the clipped hair roots. After the hair had grown long and had turned white the hairs could be identified by their cut ends. In many of the hairs the kempiness did not extend all the way down to the skin, and it may be that the degeneration started from the cut end.

White Star on Forehead

This is commonly present. Mr. D. A. MacLulich gave the writer figures for 22 hares, of which 12 showed definite white spots. Of 12 animals examined the writer found 9 with stars. It would seem that the white star is an inherited character at the line of union on the forehead and is present in about 75% of the animals. The hair growing on the star is comparable to that on either side of the linea alba, which is albinotic also.

The Hair Cover of the Under Surface of the Feet

Five hares were clipped over one half of the body including both feet and one ear. This was done after the outer covering of the feet and ears had whitened. No growth of fur occurred on the upper surfaces of the feet and ears. This was to be expected, because when the fur is white the skin also is white and the hair roots are dead. But, peculiarly, a new growth occurred on the soles of the feet. This clearly showed that there was something different in its character, and that it grew independently of the hair on the rest of the body.

As an experiment, a hare's foot was dipped in basic fuchsin. The dye instantly colored the hair on the plantar surface a bright magenta, whereas it dyed the upper parts only lightly. Under the microscope it was observed that near the skin the hairs were medullated with a very wide cortical strip, and that farther out they were twisted spirally. Probably through wear, the cortical strip in the spiral portion becomes fringed so that it has almost the appearance of a feather. This explains why the dye colored the hairs so deeply; the deep coloration was due to the penetration of the dye into the fringe. The above also explains why the soles of the feet are differently colored at various times of the year. In the spring they are noticeably blue-black. Dr. R. M. Anderson of Ottawa showed the writer some hares' feet, of which the under surface was a canary yellow (Plate IV, Figs. 30 and 31).

Erect Winter Fur Compared to Close Lying Summer Fur

When the hares are turning white a very noticeable feature is that the dark fur lies flatter than the white. In Fig. 32, Plate IV, a dark line shows along the fur over the spine. When the skin is spread out it appears as though a depression ran along the back. Fig. 33, Plate IV shows the reverse side of Fig. 32; the pigment in the skin coincides with the dark colored hair above. In Fig. 34, Plate IV, it can be seen that the dark fur lies closer to the skin than the white. As the erectness of the winter fur is coupled with the whitening process it seemed probable that some change in the skin was the cause of the alteration. A comparison was made between the growing hair roots and those which had ceased growing. As the living roots are sharply bent just above the bulb and the dead roots are perfectly straight, it was thought that this might have something to do with the erection of the hairs. It was found that the bend in the growing root is due to back pressure caused by the hair tip pushing against obstacles as it forces its way through the skin. In proof of this it will be noted that the bend is not always present and is variable in extent. Also, acute and irregular bends may be caused by drying and wetting the skin. It was assumed that the bend had nothing to do with the position of the hair above the skin, and also, as the root was below the skin, that it could not very well affect the hair outside. To determine how long the hairs grew under the skin before emerging, pieces of skin were examined under a low power microscope, and with a needle the hairs were bent at the point of emergence. The skin was then cleared and mounted and the length of hair shaft measured from the root to the bent portion. The average length of 30 hairs was 2.2 mm., the longest being 3 mm. and the shortest 1.3 mm. The new hairs grow parallel to the skin (see Figs. 5–11, Plate I). When the hair is white the hair roots have an entirely different shape; they have a brush-like termination and form a perfectly straight line with the hair. Instead of being anchored 2–3 mm. from the exit, the roots are then only 0.4 mm. from the surface of the skin (average of 20 roots), and nothing can be seen of the root sheath except bits of tissue sticking to the hair shaft just above the root, 0.2 mm. from the frayed end. These bits of tissue have the appearance of broken attachments and are to be seen on nearly all the roots. A probable explanation for the difference in distance from the root to the opening in the skin between the living and dead roots, is in the shrinkage of both the hair root and the skin itself. It is possible also that after the emergence of the new hair it may free itself from the skin by rising after desquamation of the epidermis, aided by the natural pull of the free end of the hair. And when the skin becomes thinner and tighter as it approaches the prime condition, the hair would become still more erect.

The marked differences between the living and dead roots at the time the skin becomes prime afford proof that the erectness of the winter fur is due to these changes.

Erectness of Summer Fur

It will be noted that in Table I three summer skins with erect hair are recorded. Though erectness has not been studied in summer hair as much

as in winter fur, it would seem likely that the cause is similar in both cases. It has been noted that the roots of summer hair are close to the surface, as they are in the winter skin. The flat summer fur shown in Fig. 32, Plate IV, is still growing. At an earlier stage of growth the hair would have been shorter still and closer to the skin.

Experiments on the Clipping and Dyeing of the Fur of Hares

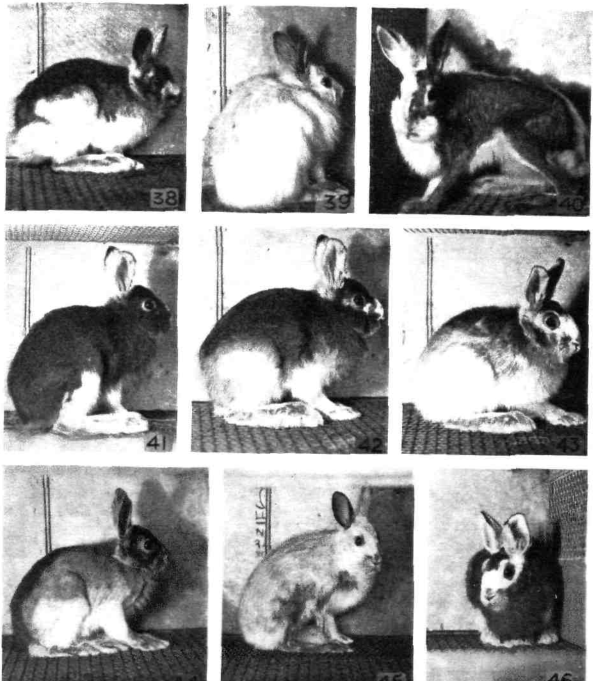
The hair of four animals was clipped over one half of the body, including two feet and one ear. Before the results of the clipping are discussed it should be stated that cutting half the fur off an animal makes it uncomfortable, and must cause a strain on the system. The results of the experiments nevertheless show that both hair and pigment will regenerate, unless the clipping is done at a time when the hair roots have ceased to function. This happened in all cases with the ears and feet and no regrowth of fur occurred except on the soles of the feet. On the legs the growth of new fur was not as complete as it was on the flanks, so it is probable that depigmentation of the skin and hair in those regions was beginning.

In three of the animals, Nos. 27, 29 and 30, a regrowth of fur from the existing roots took place. This fur was pigmented at first, and later it turned white, attaining about the same length as the fur on the unclipped side. Positive proof of this was obtained, as the ends of the hairs in Nos. 27 and 30 showed 100% cut ends. The final stage of growth was composed of a coat which was blue-black at the base, succeeded by fulvous, brown and white. The bands of color were not as uniform as on the unclipped side (Plate II, Fig. 18). Hare No. 29 grew a new coat which was blue at the base and had a buff colored layer above. The animal was killed at this stage (Plate V, Fig. 44). Above the buff were numbers of white guard hairs with cut ends. But both guard hairs and pile were present in smaller numbers than would be necessary to make the full white coat, the explanation being that the skin of the hare was beginning to whiten and the hair roots to die, especially the larger ones. A fourth hare (No. 34) was not clipped until the fur had completely whitened. Some of the blue underfur became rubbed off after clipping and the skin became bare in places. No regrowth of fur took place, except in three small spots, during 51 days, after which the animal was killed.

The clipping experiments have furnished proof that blanching of the outer parts of the hair occurs in the existing hairs, seeing that they can be identified by their cut extremities. The depigmentation of the fur is due to a destructive change.

Clipping Experiments

Experiment 1. Hare No. 27, captured at Cartier, Ontario, on May 12. On September 6 showed a definite whitening on the feet and legs.
Day 1: On September 16 the hare was white along the flank and part way up the hips and shoulders. Feet and ears were white. Skin not very dark but blackish over hips and shoulders. Was clipped on this date.



FIGS. 38, 39, 40. *Hare 34. This hare clipped after turning white. Note undercoat of blue-black fur. There was no growth of fur on the clipped side for a period of 51 days, proving that once the hair roots are frayed at the ends they cannot grow again.* FIGS. 41, 42, 43. *Dyed strip on shoulder. This strip disappeared, as shown in Fig. 43.* FIG. 44. *Hare No. 29. Buff colored coat.* FIG. 45. *Hare No. 35. Note bare ear and feet; no growth of fur occurred though the rest of the body became refurred, and later turned white.* FIG. 46. *This shows the sharp line between the brown fur and the white, as the change progresses.*

Day 10: The hair over clipped area, which grew after it had been cut, appeared to be pure white, but on closer examination in a better light it was found to be decidedly brown and a line along the back was quite dark.

Day 17: The forehead was dark brown, and back dark in color.

Day 19: The right ear which had been clipped was still brown and the hair short. The clipped feet and legs were blue and the hair was short. The hair on the clipped flanks was long and brownish.

Day 29: Legs blue up to stifle, shoulder and neck on clipped side. The brown line on the back which was noticed on October 14 had disappeared. The hair now appeared white.

Result. Day 30: Hare killed on October 18. The hairs on clipped side showed 100% cut ends. The coat was about 1 cm. shorter on clipped side.

Experiment 2. Hare No. 35, captured at Cartier, Ontario, on September 6. *Day 1:* Clipped September 26 on one side, including two feet and ear. Hair brown except a little white on ears and feet. White hair on forearm and tibia. After clipping, feet blue in color. Next to back, hair blue-black, skin pink. Blue-black color round eye, except for brown ring.

Day 21: Slight growth on clipped side of brownish hair. Unclipped side changing to white.

Day 27: Blue color round eye on clipped side turning brownish.

Day 36: Turning white round eye on clipped side. On unclipped side white, extending up hip and shoulder.

Day 46: Clipped side turning white, still brownish near backbone.

Day 53: Animal whiter.

Day 70: Animal white except clipped feet and ear, which had not changed color, and there was no growth of hair.

Day 86: No change.

Result. Day 112: Animal killed on January 15. On the clipped side the hair had grown as long as on the unclipped. The feet and ear were still bare and there was no regrowth of hair. The colored bands on the clipped side were not quite regular; the brown band below the white was missing, so that the coat was three-banded—white, fulvous and blue. All white hairs had pointed ends: they were new hairs. The skin was evidently prime when the clipping was done, and a new growth began 21 days later (Fig. 45, Plate V).

Experiment 3. Hare No. 29, captured at Cartier, Ontario, on May 12. *Day 1:* Was clipped September 26 on one side, including two feet and ear. Hare brown, feet and ears white.

Day 11: Slight growth on clipped side, of brownish color.

Day 21: Clipped side buff colored, hair ½ in. long. White guard hairs showing above buff coat had cut ends.

Day 23: Hare killed. Skin blackish, with yellow line over backbone. Under the microscope it was observed that the hair roots were beginning to die, especially the larger ones (Fig. 44, Plate V).

Result. A buff colored coat grew on clipped side.

Experiment 4. This experiment was made to ascertain whether any growth of hair would occur after the skin had become prime. Hare No. 34, captured at Cartier, Ontario, on May 12; began to whiten September 6; nearly white on October 23; pure white November 1.

Day 1: November 20, one side animal clipped, including two feet and one ear.

Day 21: Blue-black fur next skin had been rubbed off in places.

Day 31: Three small spots of hair grew on leg and nose. The largest spot about 1 cm. had a growth of white hairs tipped with black.

Day 51: Animal killed. No growth of hair except small spots mentioned above. Skin prime with few small black spots where hair had grown.

Result. It is quite clear that once the skin becomes prime in the autumn a regrowth of fur will not occur after clipping (Figs. 38, 39 and 40, Plate V).

Dyeing Experiments

Experiment 5. (Aniline blue.) Hare No. 30, captured at Cartier, Ontario, on May 12.

Day 1: September 26, a portion of the flank clipped. Aniline blue was rubbed onto the hair stubs and the skin. On this date, extremities white, nose white; white extending up legs.

Day 21: Animal nearly white.

Result. Day 23: Animal killed. Skin nearly prime; hair nearly white; on clipped patch hair not quite as long as on unclipped side.

Microscopic Examination of the Hair

The color bands were rather irregular (see Fig. 18, Plate II) but a regrowth occurred, a little shorter than that on the unclipped side. The hair was largely of kempy nature. All the fibres had cut ends tipped with blue (see Fig. 36, Plate IV), the remainder being white. It was evident that the dye which had colored the shaft of the hair stumps had disappeared. In a few hairs which had not whitened, and consequently had not lost their natural pigment, the dye adhered to the cuticle like a coat of paint, but the coloring was not as brilliant as at the tip.

Result. This experiment shows that there was a regrowth of hair from the original roots; that the hair became banded as before. It then whitened like the rest of the animal's coat. The clipping and application of dye identified the guard hairs and pile, and, as 100% were thus marked, the evidence is conclusive that the hairs whitened after they had finished their growth.

Experiments in which the Brown Hair was Marked with a Dye Before the Autumn Change to White

It was expected that the dyed hairs would remain colored after the surrounding fur had turned white. Dr. R. O. Hall of the Ontario Research Foundation kindly supplied the dye used in the experiments, namely, Clothfast Red GR. The dye was applied cold. The hair was combed over a card

and the dye applied; the hair was sponged with water afterwards to prevent the skin being burned. The dye colored the outer guard hairs and pile, the two brown layers and a little of the blue zone.

Experiment 1. Hare No. 28, received from Cartier, Ontario, on May 12. *Day 1:* On September 16 two strips on hip and shoulder were dyed red. On this date the feet and ears were whitish; the rest of the body was brown.

Day 12: White extending on legs.

Day 26: White extending into dyed area. The hairs had the appearance of whitening under the dye.

Day 33: Dye almost gone. Animal whitening fast except for brown areas on backbone and head.

Day 34: Hare killed on October 18.

Result. As the dye had apparently disappeared, it was surprising to find that it was still present in its original coloring below the outer fur. The outer hairs, both pile and guard hairs, were white except for occasional hairs which had not yet lost their own natural pigment, and on such hairs the dye still adhered externally. The under fur, which normally retains its pigment when the outer coat whitens, was colored a vivid red. This would indicate that a hair which contains its pigment may be dyed, but that as soon as the hair whitens it will lose its own pigment and the dye as well. This implies that the dye had an external effect only, and had not penetrated into the hair. In the normal whitening of hair, it has been shown that a shiny cortical strip on the outside of a hair will produce a white coloration, if there is no colored material below it. In addition to this the whitened hairs showed evidence of degeneration as described in the section on winter coloration.

Experiment 2. Hare No. 34, received from Cartier, Ontario, on September 26. *Day 1:* Strip dyed October 5 on shoulder and one hind foot. Hare had white ears and feet, otherwise brown in color.

Day 26: Nose turning white; depigmentation extending up legs.

Day 44: The dyed area had whitened for about ½ in.

Day 55: The white area had extended, surrounding the dyed strip.

Day 61: The dye had practically disappeared.

Day 71: As the dark strip along the back had not turned white, the ends of the outer hairs were clipped off and dye applied to a strip about 2½ in. wide. Dye was also applied to a patch of newly whitened hair on the flank. The hind foot which was dyed on Day 1 remained pink in color all through the experiment.

Day 92: Hare killed.

Result. The dyed patch on the shoulder had disappeared externally, as in Experiment 1 (see Figs. 41–43, Plate V). The underfur was still well colored with the dye. The patch of white hair which was dyed on Day 77 remained colored, as also did the foot which was dyed on Day 1. The clipped fur along

the back had whitened and at the clipped ends the dye had penetrated little, so that each hair had a red tip (Fig. 37, Plate IV). The results confirm the previous results of clipping the hair before it turned white.

Discussion

As mentioned previously, the principal point which the writer desired to confirm was, as indicated in his previous paper (10), that the blanching of the fur in wild hares is due to a degeneration and partial depigmentation, and not to a replacement by pure white hair. The experiments show that the whitened fur still contains pigment at the tip and at the base, consequently it could not have grown in as completely white hair. The last of the fur to whiten is that on the back, where it appears isolated from the rest of the coat, and there is a delicate gradation from brown to white, with no appreciable shedding. The lengths of the brown and the whitened hairs are equal (see Fig. 17, Plate II). The results of the experimental clipping of the animals also furnish proof that the regrowth of hair whitened like the rest of the coat and could be identified by the cut ends. The results of the dyeing experiments furnish the same proof, the ends of the whitened hairs being marked by dyed tips.

The clipped hairs also showed color bands below the white tips, proving that pigment was being produced during their growth. The underfur stays pigmented throughout the winter in Ontario hares, so that the color bands remain. The only noticeable effect on the underfur caused by the whitening of guard and pile hairs is that they lighten the color of the bands a little. The white appearance of the guard hairs is deceptive because the parts of these hairs which are in the under coat appear whiter against the dark fur than they really are. These guard hairs contain large melanin granules, and therefore are not pure white. The pile hairs in the underfur are not visible because of their thinness.

Unexpected confirmation that the bleaching of the outer fur is due to an alteration in the nature of the hairs was obtained by dyeing them before they whitened. The dye disappeared as the hairs changed color, proving that there was a definite alteration in the cortical part of the hair. The underfur, which retains its natural shape and pigment, held the dye.

There is a close connection between the depigmentation of the hair roots and the bleaching of the hair above, and as soon as the roots have lost their pigment they cease to grow. It should be added that the white hairs are plainly dead and degenerating also; if they were of new growth, as some writers think, it would be expected that they should resemble new, well-shaped white hairs of the albino type. The writer believes, however, that a pigmented animal, such as the snowshoe hare, could not grow pure white hair except in regions such as the linea alba, or following some form of injury. Since the writer has found a "prime" condition of the hair roots in summer, similar to that occurring in winter skins, it naturally brings up the question why the hair whitens at one season and not at another. This cannot be

answered until more is known about the process underlying the change. But it can be pointed out that the bleaching occurs only in two kinds of hairs, the guards and the pile. The underfur and the tips of the ear retain their pigment, though both lose the pigment in their roots. Finally, it should be mentioned that the fur attached to the prime summer skins, such as were collected during the past season, must be shed before the autumn change to white. In all the skins examined during the change, the hair roots were in process of depigmentation, and this fact confirms what has been already stated, that the hair on these animals was of recent growth, because it is certain that once the coat has attained its full development the hair roots die and must be replaced by a new growth.

Since 1929 very few comments on the writer's work appeared, until 1932 when several articles were written by Grange (6, 7), Boyd (2–5) and Gunn (8, 9). Gunn was in general accord with the writer's ideas on the whitening of the autumn fur. Grange differed from the main conclusions. Grange's two papers deal with the color phases in hares and also with protective coloration. He adheres to the idea that new growth of variegated, white tipped hairs replaces the summer coat. He says, "It is not a question of a black pelage, for example, being replaced by a white one but rather involves the replacement of one variegated pelage by another variegated pelage." If Grange's explanation is correct, that a new growth of white tipped hairs takes place in the autumn after the moult, it implies that the new hairs start growing as white hairs, and if so, we should see pure white underfur prior to the outward whitening of the animals. Grange's explanation of the change to white is unsupported either by figures of the hairs, or by mention of the hair roots or the skin in which such changes come.

Boyd (5) makes the following statement: "The seasonal whitening which takes place in such animals as *Lepus americanus* has been ascribed to an acute and complete bleaching of the pigment granules themselves (Hadwen, 10) and not to the shedding of the coat as a whole. Atmospheric oxygen is powerless to produce such an exaggerated change, and since there is no known connection between the living body-cells and the wholly dead keratinized cells of the fibres, it is difficult to reconcile these facts with the theory. It is more probable that the whitening is due to the gradual replacement of the old coloured coat by a new white one without mass shedding of the coloured coat as a whole." The writer wishes to correct the opening sentence in which he is misquoted, as he did not state that an acute and complete blanching of the pigment granules took place, but that the whitened hairs still contained pigment in certain areas and that they were not of new growth. Another conclusion of Boyd's which may be attributed to the writer is that atmospheric oxygen produces this exaggerated change. It is well, therefore, to state that there was no suggestion of this sort in the writer's paper (10). As to the replacement of the colored coat by new white hair, Boyd proposes this solution of the problem without giving any experimental support of the idea.

Acknowledgments

The author wishes to acknowledge his indebtedness to Dr. H. B. Speakman, Director of the Foundation, for advice and helpful criticism; to his colleague Mr. A. M. Fallis for the drawings in this article, and for willing and valued help in the experiments; to Mr. Elmer Craine, Messrs. J. Larkin and J. Bailey for their many courtesies and help in securing live hares which made the investigation possible; to Dr. R. Law and Mr. D. A. MacLulich for fetuses and hare skins; to colleagues in the Foundation for many helpful suggestions; and to Mr. J. E. Pritchard, animal keeper, who has been successful in keeping the hares healthy in captivity; and to Mr. R. A. Stockhill for the photos in Plate II.

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ADDITIONAL OBSERVATIONS ON SOME UTRICULARIACEAE[1]

By Francis E. Lloyd[2]

Abstract

In previous papers by the author several types of traps of *Utricularia* have been described; in the present the trap of *Biovularia* (very closely akin to *Utricularia*) is described and found to be a distinct type, combining in itself characters of the two previously known, described traps of the types *vulgaris* and *purpurea*. In the species *U. tubulata* v. Muel., which is a freely floating form not suspected previously of having a trap of structure other than that of *U. vulgaris*, it is shown that it conforms with that of the Australasian "land" species *U. manziesii*, *dichotoma*, etc. *U. tubulata* is an inhabitant of N. Queensland also.

In place of the rather easily accepted idea, expressed even by Goebel (1) that the floating forms are all alike, we now know of three distinct types, represented by the species, as types of their groups, *U. vulgaris*, *U. purpurea* and *U. tubulata*.

Since the publication of my recent paper, being the Presidential address (7) before the Section of Botany, British Association for the Advancement of Science, Leicester meeting, 1933, opportunity better than that previously available has been afforded for the study of material of the genus *Biovularia* and of a species of *Utricularia* collected by W. E. Armit in the mountain swamps about Rockingham Bay, Queensland, and described by F. v. Mueller as *U. tubulata* (9). True, this material was in the collections of the British Museum at South Kensington, and herbarium specimens are at best not very serviceable for such purpose as the present; nevertheless I have been able to make out quite clearly certain critical points of structure which justify the following notes, awaiting, with the favor of fortune, adequately preserved specimens of these interesting plants. I am indebted to the Authority of the Science Museum Herbarium for permission to take minute but, as the event proved, sufficient material for my present purpose.

Dried specimens of the traps of these plants do not recover their original form, so far as my own methods of treatment go; but traps which have by chance been pressed laterally and symmetrically are often sufficient to enable one to identify them as belonging to one or other of the types already described by me (2, 3, 7), or to permit the recognition of an additional type, as in the case of *Biovularia*. It cannot be claimed that the descriptions below can be pushed too far in the matter of detail, only that they are substantially correct.

What has been said in previous publications (2, 3, 7) will enable the interested reader to follow these notes without a lengthy recapitulation of the general characters of the *Utricularia* trap*. Those with which we are here concerned are the form and anatomy of the door or valve and of the threshold. If these can with certainty be characterized, even though not exhaustively described, it is sufficient. It is, of course, important to observe the velum,

[1] *Manuscript received March 16, 1934.*
Contribution from the Department of Botany, McGill University, Montreal, Canada.
[2] *Macdonald Professor of Botany, McGill University.*
See particularly References 2 and 3.

but this is rather too much to expect unless properly preserved material is at hand. That the velum is present, however, may be inferred from the histology of the pavement epithelium of the threshold.

Utricularia tubulata

This is a submersed floating plant of the general appearance of *U. vulgaris*, judging from the herbarium specimens as they lie before the eye, but from the description of F. v. Mueller (9), "The leaves capillary-linear, segments of the leaves verticillate and with many ascidia", it may be inferred that the plant more resembles *U. purpurea*. So far as I can make it out, however, the traps are supported on lateral stalks and this does not accord with the latter. Since it is a floating form, I had earlier rather supposed it to resemble, in the form of its trap, *U. vulgaris*, judging from a fragment of material kindly sent to me by Dr. A. B. Rendle. Additional material (three traps only) showed that this supposition was wrong; nor does the trap resemble that of *U. purpurea*, but most interestingly the form aligns itself with that of the traps of certain other Australasian species, all, however, so far as we know, of the so-called terrestrial habit. From the point of view of the questions of the origin and geographical distribution of the Utricularia, this fact is of paramount importance.

The alliance of *U. tubulata* with the type of *U. hookeri* (3) or of *U. monanthos* (7), etc. is first of all indicated by the form of the trap and its appendages (Figs. 1, 2; Plate I—1-4). In lateral view it is oblique and semicircular with a longish stalk at one end. At the other end arise three long unbranched appendages, one and one-half times as long as the trap. One of these is central, arising from the middle point of the overhang of the entrance, and corresponds to the rostrum found in *U. monanthos*, *U. menziesii*, *U. dichotoma*, etc., but is enormously elongated. On each side, above the entrance, is another quite similar appendage, so that the trap has two long "antennae" and a rostrum, making three processes extending forward for the distance indicated (Plate I-1). There are no other appendages, in this departing from the type *U. monanthos*.

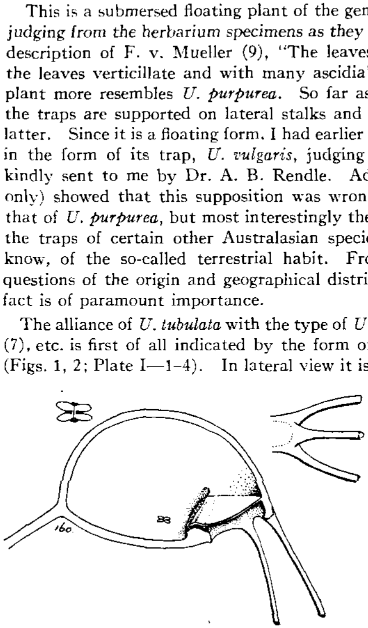
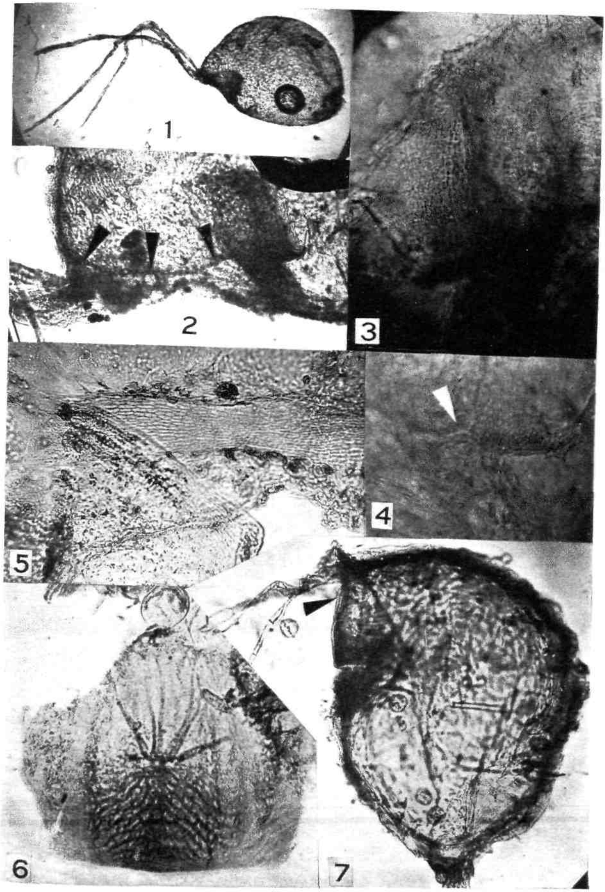


FIG. 1. *Diagram of the trap of U. tubulata based upon an interpretation of Figs. 1-4 in Plate I. Upper left, a quadrifid trichome; right, the front of the trap from above.*

The profile of the door and threshold are revealing. This can be seen in a symmetrically compressed trap after clearing (Plate I-2; Fig. 1). The door makes a long arch, curving downward and backward in a manner quite as I have represented for *U. dichotoma*, etc. It can be seen (in sagittal optical



FIGS. 1–4, *Utricularia tubulata;* FIGS. 5–7, *Biovularia.*

FIG. 1. *Lateral view of the trap, with long rostrum and two antennae.* FIG. 2. *Entrance of the trap, showing the silhouette of the door (indicated by three pointers) and the threshold on the right.* FIG. 3. *One-half of the threshold, the forward zone of which is overlain by the edge of the door, seen as a veil, the edge coming up to the inner angle of the threshold. The right and left orientation of this picture is the opposite of that of Figs. 2 and 4. From an infra-red negative.* FIG. 4. *Detail of Fig. 2 showing something of the structure of the threshold (optical section) and the edge of the door (indicated by the pointer) out of position through shrinkage. Infra-red negative.*

FIG. 5. *Half of the threshold and of the door-edge of Biovularia.* FIG. 6. *The door from in front.* FIG. 7. *The entire trap, lateral view. The tripping bristles lie against the upper half of the door and are indicated by the pointer.*

section) to be of uniform thickness, true of no other type of trap, and to meet the threshold at a very narrow angle (7). The latter is evidently ridged (Plate I—3–4), and it can be inferred that the lower free margin of the door (Plate I–4) overlies the elevated middle zone (ridge) of the threshold. This structure is, however, sadly contracted and distorted in the specimen, but its expectedly more firm and resistant marginal zone could be clearly seen in an infra-red negative (Plate I–4). Another trap afforded on dissection a fairly clear picture of the pavement epithelium for the extent of one-half the length of the threshold (Plate I–3). This most resembles that of *Polypompholyx* (Fig. 2) but that need give us no cause for pause, as this plant conforms with *U. menziesii* in the general character of the entrance mechanism —that is to say, the threshold is not the very wide affair (in the fore and aft

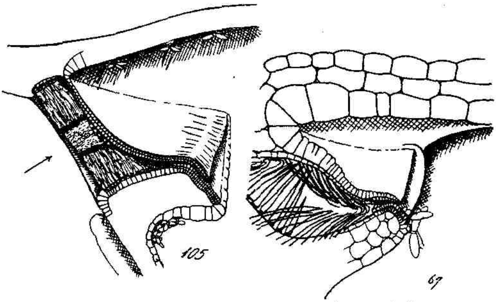


FIG. 2. *Left, U. hookeri; right, Polypompholyx multifida. For comparison with Fig. 1.*

sense) of *U. menziesii, U. monanthos,* etc. for the silhouette (Plate I–2) indicates a narrower platform in front of the zone of actual contact between the door and the threshold. It is from this platform that the velum springs, but, though I have not been able to see it, I have been able to get some indications of a velum *above* the entrance, again as in *U. menziesii,* etc. The velum would seem to be of the circular kind of this species and its allies (Fig. 2–105).

The evidence observable therefore supports the view that the trap of the floating species, *U. tubulata,* conforms to a strictly Australasian type to which belong also *U. menziesii, U. dichotoma, U. volubilis* and *Polypompholyx* (two species), so far as my observation goes. Among the total of the species of the floating submersed habit, therefore, we can recognize at this time three types, represented by *U. vulgaris* and its allies, the *U. purpurea* group, and *U. tubulata,* which may not be unique, however.

It may be noted here that the character chosen to support the specific name is the inflation of the flower-scape, which we may suppose makes it

act as a spar-buoy, helping to support the flowers above the water surface One other species, *U. warmingii* of the New World, has this feature, as described by Merl (8).

The interior surface of the trap is armed with many quadrifid trichomes, the arms of which are short and thick and lie parallel instead of spreading (Fig. 1). I can find no bifid trichomes in the usual position, that is on the inner surface cf the threshold, but this may be owing to failure of preservation.

Biovularia

This is a monotypic genus, diagnosed by Kamienski, inhabiting eastern tropical America. A submersed floating plant, it has the habit of *U. vulgaris* or *U. purpurea*, with a leaning toward the latter.

The trap stands upright on its stalk and shows but little, if any, obliquity. In lateral view, it is broadly pear-shaped, somewhat flattened on the side of the entrance (Fig. 3; Plate I–7). Each of the upper angles of the entrance bears an antenna which resembles that of *U. vulgaris* but extends forward with a downward curvature as in *U. gibba*.

The door, though conforming to a more fundamental type which would include that of *U. vulgaris*, differs so much from this as to set it apart as unique. Its tripping bristles are five or six in number (instead of normally four in *U. vulgaris*, etc.) and are inserted at, or just below, the middle point of the door as seen in sagittal section, in this resembling *U. purpurea* and not *U. vulgaris*. The lower half of the door in its middle reach is therefore the middle piece and it is uniformly thick and massive. The thickness is fairly uniform and it has curvatures which, if slight, are characteristic and can be better appreciated by a glance at Figs. 3 and 4–*B*. The upper half is the outer hinge, and is thinner with a slight S-shaped curvature, the concave element being the more pronounced. It almost duplicates the condition of *U. purpurea*. The tripping bristles are so inserted that their bases, in contact with each other, form a crowded transverse row. While the recognition of a central hinge is difficult if not impossible in dried material, even after treatment and clearing, the fact that in this kind of material the bristles are always laid up against the outer hinge (Plate I–7) indicates a ready movement in this direction, at least, in the living trap.

FIG. 3. *Diagram of trap and of front view of the door (right) of Biovularia violacea Kam. The middle piece of the door is below the tripping bristles and is indicated by a surrounding broken line.*

Their mode of insertion and form are quite as in *U. vulgaris* and there is knob-like protuberance as in *U. purpurea.*

When the door is examined *en face* (Plate I–6), it is seen that its anaton is practically identical with that of the door of *U. purpurea* (5) in regard the proportions and directions of the component cells. As just mentione however, there is no knob, and the beading along the free edge of the doc seen in *U. purpurea*, is also absent. The middle piece is indeed somewh

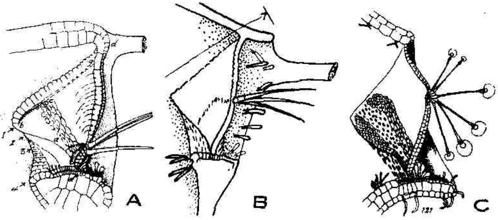


FIG. 4. *A, U. vulgaris; B, Biovularia; C, U. purpurea, for comparison. In A, in a strictly sagittal section, only two bristles would show.*

more massive, and is applied to the threshold in a wider angle than in tha species, having therefore a more direct thrust against the pavement epithe lium (7), so that the velum readily adjusts itself without any further as sistance.

The threshold is of the type in *U. vulgaris* (4) in shape and in structure c the pavement epithelium (Plate I–5). A slight matter of difference is foun in the fact that it lies somewhat as in *U. subulata* (Fig. 5) or in *U. resupinat* (2) and rather more forward than in that species, so that, as mentione above, the thrust of the door is approximately normal to the plane of th pavement epithelium. The inner wall of the threshold bears a few bifid (? trichomes, while the inner wall surfaces bear quadrifids with rather lon spreading arms. The number of these hairs is small in contrast with th *vulgaris* or *purpurea* type.

It will be seen that the trap of *Biovularia* is a curious mosaic of structure apparently derived from *U. vulgaris* on the one hand and *U. purpurea* on th other (Fig. 4). While the former is cosmopolitan, it has closely relate American species, and the latter is confined to the New World. If one dare to speculate beyond the evidence, one might make out a case for the origin o *Biovularia* as a hybrid. At all events, the facts as known are suggestive.

Briefly summarizing, it has been shown that the trap of *U. tubulata*, a submersed floating form, conforms to a type which is confined to Australasia

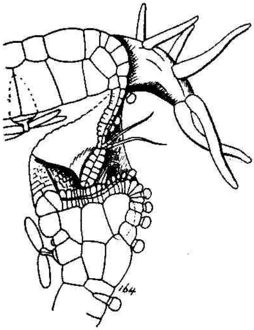


FIG. 5. *U. subulata. Diagrammatic representation of a sagittal section of the entrance. (Preserved material kindly supplied by Prof. J. C. Th. Uphof.)*

of which *U. monanthos*, *U. menziesii*, etc., have been taken as exemplars. The relation in phylogeny of these is analogous, one may argue, to that between the *U. vulgaris* type and certain terrestrial forms, such as *U. subulata* (Fig. 5); that is, there are corresponding terrestrial and submersed forms which can readily be conceived as having followed parallel courses of evolution.

Biovularia offers a trap with such structure that it stands alone as unique and therefore adds a type to the list already established in my previous publications and making a fourth member of the floating series above mentioned. It appears to be a mosaic of characters of two types, exemplified in the type of *U. vulgaris* on the one hand and of *U. purpurea* on the other.

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A BIOMETRIC STUDY OF THE CULMS OF CEREALS AND GRASSES[1]

By Henri Prat[2]

Abstract

Many attempts have been made to use absolute biometric values as accessory specific characters in cereals and grasses. The present note is an attempt to express by plain graphs not only the absolute average lengths but also the relations of length between the internodes of the culm.

In some cases, the graphic representation leads to a simple mathematical formula; in others this is not possible, but the shape of the graph gives interesting information on the role played by the young inflorescence during the growth of the culm. Sterile or, on the contrary, hypersexual culms have the history of their youth inscribed in their adult structure.

The observations in the present paper will be connected with a larger study of the conditions of growth and mode of differentiation of the plant tissues. Relations with the peculiar structure of the epidermis of the culm are discussed.

I. Increasing Size of the Internodes of a Cereal Straw (Biometric Gradation)

It is a conspicuous fact that the internodes of cereal straw increase regularly in size from the base to the top. This peculiarity seems to be an expression of the special polarity of the stem, caused by the presence of the ear at its apex; that is to say, a biometric form of the *gradation* (3, p. 199) noticeable also in morphological, anatomical and histological features (5, p. 165).

Let us consider, for example, a plant of Einkorn wheat (*Triticum monococcum*). The length of the internodes of the 10 straws forming the tuft are shown in Table I.

TABLE I

Lengths of internodes of the 10 straws forming a tuft of Einkorn wheat

Internode	Straw										Average lengths, cm.
	I	II	III	IV	V	VI	VII	VIII	IX	X	
	Length of internode, cm.										
7	436	472	190	357	193	294	313	162	340	359	311.6
6	209	221	132	177	124	171	177	99	203	210	172.3
5	95	90	56	82	60	95	91	73	88	100	83
4	25	25	38	40	24	60	56	65	50	64	44.7
3	10	12	15	9	4	38	31	23	16	25	18.3
2	5	—	5	5	—	14	6	5	5	10	5.5
1	—	—	—	—	—	8	2	1	—	4	1.5
Total length	780	820	436	670	405	680	676	428	702	772	636.9

[1] *Manuscript received March 13, 1934.*
Contribution from the Department of Biology, University of Montreal, Montreal, Canada.

[2] *Director of the Department of Biology, University of Montreal.*

For purposes of comparison the top internodes of all the stems are given the number 7, this being the number of elongated internodes on each of the principal culms. The second internode from the top is number 6, and so on. *On this basis the sizes of the successive internodes are plotted on Fig. 1 and the lines join the points representing the internodes of each* culm. While there are irregularities, notably in the cases of minor culms III, V and VIII, the broken lines follow a concave and upward path. The curve *M* representing the average size of the internodes is more regular than the curves for individual stems since the small differences due to the conditions of growth peculiar to each stem are levelled out.

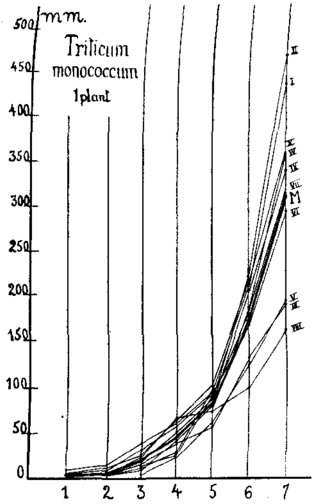

Fig. 1. *Relation between the lengths of internodes of a plant of Einkorn wheat (10 straws) and their numerical rank, plotted according to the ordinary system of co-ordinates. The heavy line M represents the average values.*

Many attempts have been made to express by a simple formula the phenomenon of the increasing size of the internodes. Novacki* states that "the length of each internode is the arithmetic mean of the lengths of the internodes immediately above and below it". Lloyd (2, p. 3) says "the terminal internode in wheat is 50 per cent of the whole straw in length and over twice that of the subterminal internode". But those expressions have only the value of a practical rule, which does not always coincide with reality, and cannot lead to an interpretation of growth.

An attempt has been made here to develop a more significant expression of the biometric gradation of internodes and this has, in some cases, been successful.

II. Mathematical Expression of the Biometric Gradation in Some Straws

In Fig. 2 a logarithmic scale has been used, the ordinates representing length of internode. The curve in Fig. 2 *A* is based on the same data as curve *M*, Fig. 1, and represents the averages for the 10 culms listed in Table I. Fig. 2 *B* is based on 20 culms from two plants of the same strain of *Triticum*

*See References (4, p. 63) and (1, p. 67).

monococcum. The obvious tendency of the points to fall on a straight line, most marked in Fig. 2 *B*, indicates that chance is not an important factor.

The equation expressing this relation is:

$$\log Y = K \quad \log n + \log A \quad (1)$$

where Y is the length of an internode; n, its numerical order; K, the slope of the straight line; and A, a constant. Therefore:

$$Y = A \; n^K, \qquad (2)$$

which is an expression of the length of the internode as a function of its numerical rank.

To obtain the value of K, we can substitute the lengths of two internodes, for example, 2 and 7, in the formula:

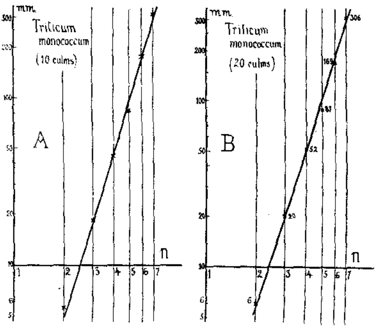

FIG. 2. *Relation between the lengths of internodes and their numerical rank, plotted on a logarithmic scale. A. Average lengths of the internodes of the 10 straws considered in Fig. 1. B. Average lengths of 20 culms of two plants.*

$$K = \frac{\log Y_7 - \log Y_2}{\log 7 - \log 2},$$

from which we obtain (from data in Fig. 2 *B*)

$$K = \frac{\log 306 - \log 6}{\log 3.5} = \frac{2.48572 - 0.77815}{0.54407} = 3.135.$$

The exponent K gives the slope of the curve, that is to say, the *rate* of increase in size from one internode to the next. We can take it as an expression of the average *biometric gradient* of the culms.

A is then obtained from Equation (1).

$$\log A = \log 306 - 3.135 \log 7.$$

The length of a given internode, Y_3, may be calculated as follows:

$$\begin{aligned}\log Y_3 &= 3.135 \log 3 + \log 306 - 3.135 \log 7\\ &= 3.135 (\log 3 - \log 7) + \log 306\\ &= \log 306 - 3.135 \times 0.3680\\ &= 2.48572 - 1.15368 = 1.33204,\end{aligned}$$

from which, $Y_3 = 21.8$. Actually $Y_3 = 20$. Similarly

Y_4, calculated, $= 52.95$. Actually, $Y_4 = 52$.
Y_5, calculated, $= 106$. Actually, $Y_5 = 87$.
Y_6, calculated, $= 188.8$. Actually, $Y_6 = 169$.

The graphs obtained for isolated straws are far less regular than those obtained with mean values for a large number of straws. The individual fluctuations of the internodes may reach 50% of the average value and sometimes a given internode may even be smaller than the internode immediately

below it. Therefore the formula $Y=An^K$ gives in individual cases only a rough approximation. However, the comparison of many straws belonging to the same plant may give rise to interesting information, and the most valuable element of the formula is perhaps the expression of the gradient K.

It will be observed that the principal stem has the greatest slope and highest exponent K; then come the secondary stems; then the tertiary stems with the lowest value of K, as far as it is possible to consider a direction for the line joining the irregular points obtained with the small straws.

III. Comparison of Some Cereals and Grasses in Regard to the Biometric Gradation

A. *Cereals*

The mathematical relation shown above does not apply to every case. However, the writer has found it to be an approximate expression of the biometry of straws in many cereals. For example, in specimens of Spelt (*Triticum spelta* L.), very regular in shape, as many as six points were obtained in a straight line (Fig. 3 *A*), a fact which excludes, as in the case of *T. monococcum*, the hypothesis of the effects of chance.

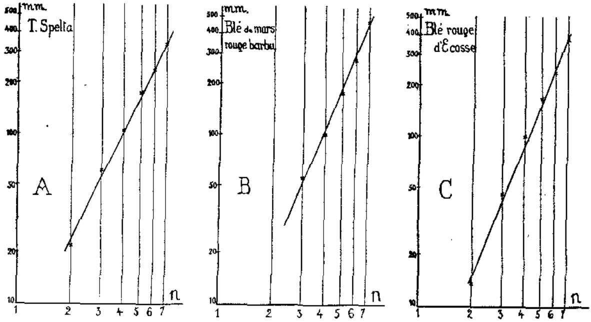

Fig. 3. Logarithmic relation between the lengths of internodes (average values) and their numerical rank. A. Triticum spelta—11 culms. B. Blé de Mars rouge barbu (T. vulgare ferrugineum)—12 culms. C. Blé rouge d'Ecosse.

With some wheats of the group *T. vulgare*, for example, on "Blé de mars rouge barbu"; (*T. vulgare ferrugineum* Korn.), Fig. 3 *B*, and on "Blé rouge d'Ecosse" (*T. vulgare milturum* Perc.), Fig. 3 *C*, the law can be verified in a satisfactory way. In general, the five upper internodes are elongated and can be used for the establishment of linear graphs. But in other varieties or species the conditions are entirely different. Consider, for example, the graphs for a sample of Miracle wheat (*T. turgidum mirabile* Korn.), Fig. 4 *A*. In this case the internodes 2, 3, 4, 5 and 6 give points regularly enough disposed on a straight line. But the last internode (No. 7) is placed distinctly

above the line traced by the inferior internodes. It seems that, in this case, a hypertrophic and abnormal elongation had affected this upper internode during its growth. We can attribute this fact to a sort of "hypersexuality" of the plant. A similar peculiarity was found with wheats of the group "Amidonnier" (Fig. 4 *B*), (*T. amyleum* Ser, *dicoccum* Schübl), and it seems rather frequent in the species *turgidum* and its varieties. Analogous facts appear in the study of annual wild grasses.

It is clear that, even when it is impossible to recognize a regular mathematical rule, a logarithmic graph can give interesting information on the species examined. Thus, when the plant is grown under sufficiently normal conditions, and when the average is based on a number of straws sufficiently large to eliminate individual fluctuations, the *shape* of the graph can express characteristic features of the plant. The shape of the curve would be more useful than the absolute measurements which are often used as systematic characters (3, 4); since absolute sizes are more directly affected by external conditions, whereas the shape of the graph expresses, to a great degree, the reciprocal internal influence (interactions) of portions of the same stem during growth.

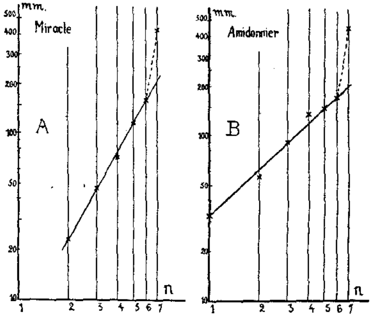

FIG. 4. *Logarithmic graph—A. Miracle wheat. B. Amidonnier.*

Other cereals than wheat, *e.g.*, barley (*Hordeum*) show biometric characters with the same types of gradation as the wheats mentioned.

B. *Wild Grasses*

Wild species of grasses show characters similar to those of the cultivated cereals.

In the logarithmic graph for a straw of *Poa nemoralis* (Fig. 5), the seven upper internodes give points in a straight line, a good verification of the formula. The aerial and reproductive parts of its stem are thus built according to the same general rule as a cereal straw. But it is worthy of notice that, in the perennial species, the basal part, which is characterized by the presence of lateral shoots and short internodes, shows no definite law of length. In this inferior vegetative portion, the elongation is governed only by the antagonism of growth between internodes and shoots, by the importance of rooting of the nodes, etc.

Regularity of shape allowing the detection of a mathematical law is subject to the condition of *absolute freedom* of the axis; that is to say, the total absence

of shoots or roots which introduce a disturbance in the adduction of sap and cause the irregular growth of meristems.

Of course, in a cereal, the basal internodes, in the tillering part of the stem are also absolutely irregular and are therefore in the same class as the rhizomes of a perennial species.*

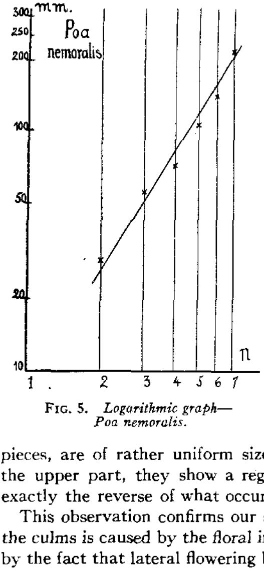

FIG. 5. *Logarithmic graph—Poa nemoralis.*

C. *Sterile and Perennial Stems*

A peculiarly interesting case is presented by the sterile shoots of some grasses. When a stem has no ear, or more generally no inflorescence, at the apex, the regularly increasing size of internodes described in preceding cases is not observed. For example, in the sterile shoots of *Holcus lanatus*, the internodes near the middle are of about equal length, and there is a progressive decrease in size towards the apex.

A most striking case is given by the stems of bamboos. These big perennial straws develop their entire growth in a single year, and their apex is, in general, entirely devoid of any trace of inflorescence. The flowers appear only on lateral branches, long after the perfect consolidation of the main stem. It should be noted, also, that all the internodes of a bamboo, above the short, rooting basal pieces, are of rather uniform size in the middle of the stem. Moreover, in the upper part, they show a regularly decreasing length towards the apex, exactly the reverse of what occurs in the fertile culms previously referred to.

This observation confirms our supposition that the biometric gradation of the culms is caused by the floral influence. And further confirmation is given by the fact that lateral flowering branches of the Bambusae show, frequently, internodes of increasing size, (*e.g.*, *Chusquea quila*, *Arundinaria hindsii*). Those fertile shoots are built on the same pattern as an entire straw of an annual grass.

The writer has indicated in another publication (5, p. 304) that the main stem and leaves of bamboo are deprived of any gradation in the structure of the epidermis. Histological and biometrical gradation, both being effects of the same cause, are thus in perfect correlation.

D. *Annual Grasses*

At the other extreme of biological conditions are the small short-lived annual grasses, such as *Mibora verna*. The logarithmic graph based on this

**By basal node or internode is meant the inferior part of the plant which bears roots and is usually underground. Some of these may conform to the logarithmic relation shown by the upper ones but, as many irregularities occur, it is preferable to avoid a general statement for them.*

plant (Fig. 6) is a very simple one, because only a few internodes are somewhat elongated during the few weeks of the vital cycle of the plant. The lengths of the internodes are: 88, 5.5, 1.5, 0.7, and 0.4 millimetres. The three points given by the upper internodes are not on a straight line and the broken line of the graph is strongly curved upwards. The last internode is relatively enormous compared with the others, and we have a case resembling to some extent that of "hypersexual" wheats, such as *T. mirabile* and *T. amyleum*, where the upper part of the straw becomes hypertrophic by sexual influence.

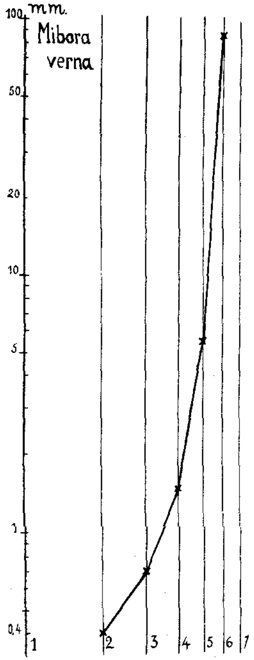

FIG. 6. *Logarithmic graph—Mibora verna.*

The influence of the maturing inflorescence is a dominant one. In *Mibora* also, and to an even higher degree, all the parts of the small and weak vegetative system are exhausted by the reproductive function, and life ends after a very short time.

The writer has pointed out elsewhere that, in *Mibora verna*, the histological differentiation of the epidermis is one of the lowest degree existing among grasses (5, p. 276). In this regard, too, *Mibora* is the opposite of the bamboo with its perennial, highly differentiated stems.*

The peculiarities of annual short-lived grasses may also be compared with differences noticed in cultures of the same cereal sown in the autumn and in the spring, *e.g.*, *Triticum monococcum*. The curves based on straws of the stock sown in the fall have slightly less slope than those based on straws of the spring stock; the exponent K of the formula is smaller.

The biometric gradation is therefore increased when the vital cycle is shortened.

Summary

1. The possibility of expressing the relations between the lengths of internodes of the culms of many species of grasses by a simple graphic representation in logarithmic co-ordinates has been demonstrated.

**The word "gradation" expresses a comparison between two parts of the same organ; the word "differentiation" expresses a property of a given area. Therefore histological gradation may, or may not, occur along a highly differentiated, or a slightly differentiated culm if both are of uniform structure.*

2. In certain cases this graph takes a linear form which may be represented by the relation

$$Y = A_1 n^K,$$

giving an approximate expression of the length Y of the internodes as a function of their numerical rank, n; K being an expression of the ***biometric gradient*** of the culm.

This relation, only roughly approximate for isolated culms of those species, becomes more accurate with average lengths calculated on a great number of straws, particularly on principal culms, in genetically pure strains, and under regular conditions of growth.

3. In other species or varieties the preceding rule does not apply. However, the logarithmic graphs afford valuable information, for the shape is often characteristic. For example, in some cases the upper internode is hypertrophied and its apical point is placed high above the line obtained with the points representing the basal internodes. This condition is shown in particular by some wheats with strong sexuality: *T. mirabile*, *T. amyleum*. Further experiments will be made to find in what measure this abnormality is an hereditary and constant character, and in what measure it can be changed by conditions of growth and environment.

4. Small short-lived annual grasses, such as *Mibora verna*, also show an hypertrophy of the upper internode. In general, the biometric gradation is increased when the vital cycle is shortened.

5. When a culm is deprived of inflorescence at the apex, as in the bamboos, it does not show the regularly increasing size of internodes (biometric gradation) characteristic of fertile culms. This peculiarity may parallel the absence of anatomical and histological gradation in those stems.

Conclusions

Culms of grasses are very suitable material for the study of the influence exercised by sexual organs on the growth of an articulated stem.

The whole history of this influence is inscribed in the structure of the adult straw and the writer intends to show subsequently the relation existing between these observations and the dynamics of growth.

Gradation means the presence of an inflorescence at the apex of the growing stem; regularity means freedom of growth of the axis. Thus, biometric gradation is only a form of the general polarity which appears in many anatomical, histological, and physiological features of the culms of grasses.

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A PHOTOELECTRIC CELL METHOD OF MEASURING THE VELOCITY OF PROJECTILES[1]

By D. C. Rose[2]

Abstract

The velocities of rifle bullets have been measured by causing the bullet to interrupt successively beams of light shining on photoelectric cells. The resulting current pulse in the photoelectric cells, after being amplified, actuates a recording device. Two methods of taking the records were used. In the first the amplified current from the photoelectric cells is recorded by an oscillograph consisting of an Einthoven galvanometer and a rotating drum camera. In the second method each current pulse from the amplifier caused by the interruption of the beam of light by the bullet actuates a thyratron so that it discharges a condenser. The current through the condenser is recorded by the Einthoven galvanometer. The time record for both methods is obtained from a valve-maintained tuning fork having a frequency of 1000 cycles per second. The output of the tuning fork circuit excites a second fibre in the Einthoven galvanometer. Four photoelectric cell stations were used in a range of about 150 ft. The first was placed about 23 ft. from the muzzle, the others at equal intervals down the range. The accuracy was such that in measuring velocities of about 4000 ft. per sec. the probable error under the best conditions was of the order of five or six feet per second.

I. Introduction

In many methods of measuring velocities of projectiles the bullet either breaks a wire screen or trips a trigger, thereby breaking or making an electric circuit by direct mechanical contact with some part of it. If screens are used these have to be replaced on the range after each round, a practice which sooner or later leads to accident where continued measurements are taken. In the case of one method often used with rifles, the necessity of replacing screens is obviated by the use of a small contactor, attached to the muzzle of the rifle, which breaks an electric circuit when the bullet touches a light pointer just in front of the muzzle. Associated with this is a piece of armor plate in the butts with protected contacts which open by inertia when the plate is struck. A great disadvantage of this method is that the position of the bullet is recorded at two points only, so only one measure of the velocity is obtained. Similar objections apply to the ballistic pendulum. A further disadvantage in any method in which the bullet interrupts an electrical circuit by cutting a fine wire or by mechanical contact with some part of the recording circuit, is that the motion of the bullet may be interfered with to some small extent by impact with whatever material it touches, or by scratches on the surface of the bullet. This applies particularly to small, very high velocity bullets being used in modern rifles.

The obvious requirements of an apparatus for measuring the velocities of projectiles are that methods should be used which would not disturb the bullet in its flight, and which would record its position at several points down the range so that velocity measurements could be made at several points on the

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trajectory for any one round. It was also desired that the apparatus should be applicable to a large range of velocities, say, from a few hundred feet per second to about 5000 ft. per sec. without any great alteration in its manipulation; and, that short intervals (not more than 50 ft.) between recording points could be used. The apparatus described below has been found to give reasonably good accuracy under these conditions, and further there appears to be no reason why the same recording parts cannot be used with any type of projectile, from rifles to heavy artillery.

II. Description of Method

In principle the method can be explained very briefly. A beam of light in the trajectory of the bullet is interrupted by it. The beam of light shines on a photoelectric cell and the change in current in the photoelectric cell is recorded. By placing a series of light beams and photoelectric cells along the trajectory the position of the bullet may be recorded at several points, and several velocity determinations made on any one round. In the present experiment four photoelectric cell stations were used. The first photoelectric cell station was usually about 24 ft. from the muzzle and the other three were spread along the range at approximately equal intervals of about 43 ft. These distances might have been shorter or longer, or more stations might have been used, without any alteration in the recording apparatus. With the range available (approximately 150 ft.) the above was a convenient arrangement for experimental purposes.

The records of the change in photoelectric cell current when the bullet interrupted the beams of light were taken by means of a rotating drum camera. Two different electrical currents were used in obtaining a record of the current pulses in the photoelectric cells. In the first the current pulse, after being amplified by a single stage amplifier, was recorded directly by means of an Einthoven galvanometer. This method was soon discarded because of its susceptibility to interference and difficulty of adjustment. In the second method a two stage resistance-capacity coupled amplifier was used to control a thyratron. The output of the thyratron was so connected that when a positive voltage pulse was impressed on the grid (when the bullet interrupts the beam of light) the thyratron discharged a condenser, the current of the condenser being recorded by the Einthoven galvanometer. Good results were obtained by this method. The time record in both arrangements was obtained from a valve-maintained tuning fork operating at 1000 cycles per sec.

General Electric photoelectric cells, Type PJ22, were used. The makers of these cells recommend that the light intensity on the cells should not be greater than that required to allow a current of 20 microamperes to flow. The ideal instrument for the purpose of this experiment would be an oscillograph which would operate directly on the photoelectric cell current without amplification. The nearest approach to such an instrument is the Einthoven galvanometer. Very sensitive Einthoven galvanometers will give a reasonable deflection with a steady current of one microampere, but in the case of

the bullet interrupting the beam of light for very short intervals of time (40 to 100 microseconds) a sensitive galvanometer fibre will not follow the shape of the pulse. The fibre is given a ballistic throw by the pulse and vibrates with its natural period. Under such circumstances the sensitivity is considerably less than under steadier current conditions. The effect of this ballistic throw on the results will be discussed later. If the beam of light representing 20 microamperes in the photoelectric cell was interrupted by the bullet, probably the 20 microamperes pulse would be sufficient to give the galvanometer an observable deflection. This would mean that the rifle would have to shoot sufficiently accurately to totally interrupt a beam of light no wider than the diameter of the bullet. Such accuracy cannot be expected at a range of 150 ft. In fact the minimum width of the beam of light required, to be certain of interrupting part of it every round with standard .303 service rifles and ammunition, was found to be about two inches at a distance of 150 ft. Hence, limiting the total photoelectric cell current to 20 microamperes, the fraction of the light interrupted by the bullet represents only about three microamps under the best conditions. A pulse of this intensity was found to be much too small to operate the Einthoven galvanometer without an amplifier. With a properly designed amplifier still wider beams of light and smaller total intensities of the light on the photoelectric cells could be used. Actually beams of light about 3 in. wide were used in photoelectric stations Nos. 3 and 4 (those farthest from the muzzle), while in the case of stations Nos. 1 and 2 it was found convenient to use beams about 1 in. in diameter. The beams used in all four stations were about one inch thick in a direction parallel to the trajectory. The effect of the thickness of the beam of light will be discussed in a later section.

Other types of oscillograph, such as the Duddel type or the cathode ray oscillograph, might have been used. The Duddel oscillograph offers no advantages over the Einthoven galvanometer. The cathode ray oscillograph obviates errors due to inertia of moving parts, but involves considerable electrical equipment and it is difficult to obtain photographic records of non-repeatable transients from it. Further, it will be seen that the greatest errors in measurement of the projectile velocities were not due to inertia of moving parts.

III. Recording and Timing Apparatus

An Einthoven galvanometer manufactured by the Cambridge Instrument Company and supplied with a double fibre head was used. One fibre was used to record the current pulses, the second to record the time between the interruptions of the beam of light. The time record was obtained from a valve-driven 1000 cycle tuning fork. The output from the oscillating circuit controlled by the fork was connected through a suitable adjusting potentiometer to the second fibre.

A pointolite lamp was used for the source of light and the images of the fibres were brought to a focus on the camera slit by the usual combination

of microprojector and a cylindrical lens (see Fig. 1). It was found convenient to place the axis of the camera in such a way that the light from the projection system was bent through approximately a right angle by two small stainless steel mirrors. By suitable adjustments of these and light stops, the position of the images of the two fibres on the camera could be adjusted so that the record of the light interruptions in the photoelectric cells and the 1000 cycle time record appeared on the film in the most suitable position for measurement.

The camera was a simple rotating drum type. The drum was 9½ in. in diameter and 8 in. wide and was so arranged that a piece of photographic

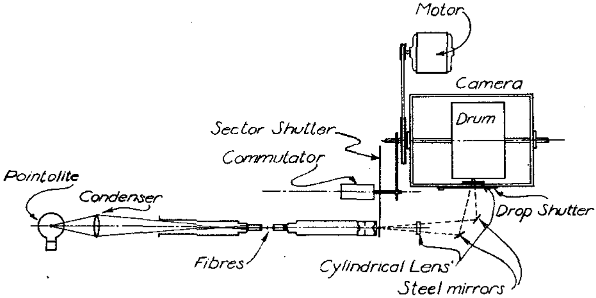

FIG. 1. *Arrangement of the Einthoven galvanometer and camera.*

film or paper 8 by 32 in. could be mounted conveniently. A shutter system was arranged so that the record was about 13½ in. long and 1 to 1½ in. wide. The camera drum could be moved along its axis and fixed to the tuning apparatus in two positions 180° apart, so that 16 separate records could be taken on one piece of film 8 in. wide. Eastman Verichrome films 3½ in. wide on which the records of six rounds could be taken were also used.

The gun was fired and the shutter released electrically. The shutter system consisted of a drop shutter and a rotating sector. The rotating sector interrupted the beam of light immediately in front of the projection microscope and actually fixed the time of the exposure, while the drop shutter immediately in front of the camera slit was used to prevent re-exposure as the sector continued to revolve. The rotating sector was driven on the same shaft as a commutator which controlled the opening of the drop shutter and the firing of the gun. This shaft was driven at the same speed as the camera drum by chain drive. The whole firing circuit was controlled by a switch which, when open, left the circuit dead. When everything was ready for a record the closing of this switch caused the camera drop shutter to be tripped and the gun fired in the proper sequence. The time delay in the shutter

and firing mechanism operation could be found easily by trial and once the proper sequence for the operation of the drop shutter, gun firing and the sector shutter was arranged, the apparatus worked in a satisfactory manner without readjustment, provided the speed of the camera was reasonably the same for different rounds.

The camera as used had to be loaded in darkness or with a photographic safe light. It was not necessary, however, to have the room totally darkened during operation.

IV. Arrangement of Apparatus with Respect to the Rifle Range

The rifle range was set up in a convenient location in the sub-basement of the National Research Laboratories in Ottawa. The ceilings are low and the space completely enclosed, so the vibrations caused by firing the rifle (though the rest was supported on a concrete pillar on bed rock) were rather severe, and it was found that the recording apparatus had to be placed some distance away from the rifle. In the process of trying out various locations of the apparatus the following arrangement was evolved and was used largely for the results given here.

The amplifier and the rheostats controlling the four lamps illuminating the photoelectric cells were placed on a table behind the butts, so no disturbance due to firing could reach it until after the record was taken. The recording apparatus (Einthoven galvanometer, timing apparatus, etc.) was located in a convenient vacant room on the storey above the range on the opposite side of the main corridor of the building. Its direct distance from the rifle rest was about 50 ft. Here it appeared to be free from disturbances due to the explosion. Actually a more practical arrangement which has been shown since to be equally satisfactory would be to locate the amplifier and lamp controls in the same room with the recording apparatus.

V. Arrangement of Lamp and Photoelectric Cell Supports

The most difficult feature of the experiment was the arrangement of suitable supports for the lights illuminating the photoelectric cells and for the photoelectric cells themselves. The photoelectric cells are microphonic and it was found that with the first mountings tried, disturbances in the electric circuit due to the explosion wave striking the cells in stations Nos. 1 and 2 completely upset the results. Trials were made with the photoelectric cells and illuminating lamps mounted in a variety of ways, both on the same support, on separate supports, rigidly supported and loosely packed in excelsior. The most satisfactory mountings were found to be those in which the illuminating lamp, lenses and photoelectric cell were rigidly mounted and as close together as was practicable. Actually three types of mountings were used, the optical systems of which are shown in Fig. 2. The lamps used were automobile headlight bulbs, 6–8 volts, 21 candle power in Types 1 and 2 and 32 candle power in Type 3.

In stands of Type 1 the lenses used were about $1\frac{1}{4}$ in. in diameter, the projection lens having a focal length of 5 cm. and the pickup lens a focal length of 10 cm., so that the beam of light spread over the plate of the photoelectric cell bringing the trajectory to an approximate focus on the plate. The lenses were about eight inches apart. The lamp, lenses and cell were mounted rigidly on a heavy piece of steel plate about two inches thick cut away as indicated in the photograph in Fig. 3. The heavier the steel plate and the more rigid the clamping of the lamp lenses, etc., the more satisfactory the results. The plate with rigid mountings practically eliminated any troublesome vibrations due to the explosion wave from the rifle, except those which were of too low a frequency to matter. The support of the heavy mounting plate appeared to be of no great importance and a light angle iron stand was used. Probably the explosion wave caused the whole mounting plate to move slightly but such a motion being slow, owing to the inertia of the plate and allowing no relative motion of the lamp with respect to the photoelectric cell, caused no trouble. Vibrations in the plate set up by the shock wave, if they existed at all, did not give any troublesome effects.

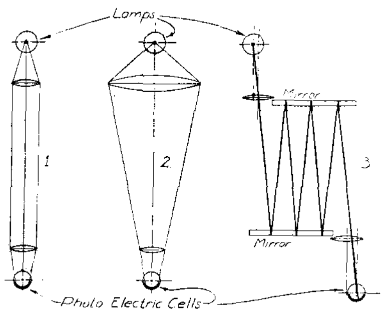

FIG. 2. *Optical systems of the lamps and the photoelectric cells.*

This type of stand (Type 1) was used in stations Nos. 1 and 2, those nearest the rifle (about 24 and 67 ft. from the muzzle). Their optical system produced a round beam of light about one inch in diameter, which was sufficiently wide for ease of alignment up to distances of about 75 ft. For greater distances a somewhat wider beam of light was required, to be certain of obtaining a record for every round. To produce such a beam an arrangement of lamp and lenses as in Type 2 of Fig. 2 was built. Here the upper lens consisted of projection lantern condenser lenses about four inches in diameter which collected the light from the lamp and condensed it to about the size of the lower lens, which was arranged with the photoelectric cell in a manner similar to Type 1. This arrangement was used at stations Nos. 3 and 4 (about 110 and 153 ft. from the muzzle) for preliminary tests and in station No. 3 only in the final layout. The lamp, lenses and photocell were supported on brackets screwed to a strip of 3 in. channel iron in a manner not shown. The beam of light was stopped down at the upper lens so that it was rectangular in cross section, the long dimension being perpendicular to the trajectory. The cross

section of the beam at midpoint between the lenses was about three inches by one inch. This type of support is not recommended as Types 1 or 3 are much more satisfactory.

In the case of the third type of lamp the effective recording area (*i.e.*, the area normal to the trajectory any part of which is effective in producing a record when penetrated by the bullet) is increased by using a relatively narrow beam of light reflected several times by two flat mirrors as indicated in Fig. 2. To make this method of measuring velocities practicable it is essential that its utility is not spoiled by the necessity of very fine adjust-

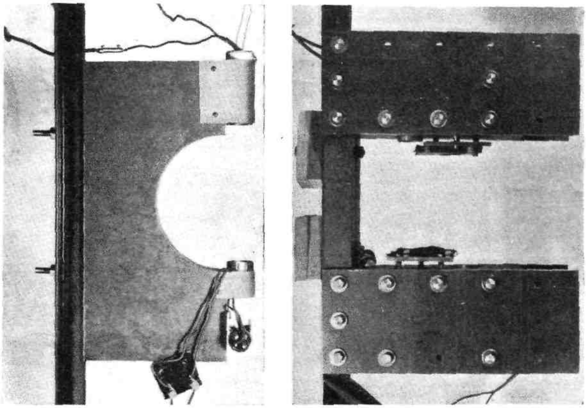

FIG. 3. *Photographs of the lamp and photoelectric cell mountings. Type 1, left; type 3, right.*

ments of the rifle rest, or, if it should be used for testing ammunition the exact ballistics of which is uncertain, the effective recording area of photoelectric cell stations placed some distance from the rifle must be sufficiently large to include all but really wild rounds. The lamp and photoelectric cell stations must also be so designed that they are not influenced by surrounding illumination, or can be protected from it easily. Further, the application of this method of measuring projectile velocities to larger projectiles than rifle bullets is limited only by the feasibility of producing suitable light beams and photoelectric cell mountings. With these points in view the lamp and cell mounting of Type 3 was designed. In order to make a large effective recording area the beam of light (roughly parallel and about $\frac{3}{4}$ in. square) was reflected six times as indicated. The mirrors were about six inches apart and the reflections were so arranged that an area about four inches

wide and over five inches high was completely illuminated. The thickness of the beam in a direction parallel to the trajectory was about $\frac{3}{4}$ in.

The support was made of heavy 4-in. channel iron and $\frac{1}{4}$-in. boiler plate, bolted together and the lamp and photoelectric cell were rigidly mounted. The design was such that the photocell and lamp were completely enclosed in the steel framework so that only light coming directly from the upper mirror could affect the photoelectric cell. Fig. 3 shows a photograph of the stand. The vertical channel iron on the right is removed for adjustment. In the results reported here this piece was omitted as the stand was sufficiently rigid without it. A mounting for lamp and photoelectric cell of this type should be suitable for artillery of almost any class if built in an appropriate size to scale. For instance, if such velocity measuring equipment were desired for a field gun of three inches calibre, a stand similar to the above would be built 12 times larger in all dimensions normal to the trajectory (it should not be necessary to increase the cross-section dimensions of the steel sections by anything like this factor). The effective recording area would then be about four by six feet. This should be ample for velocity measurements at reasonable experimental ranges.

If permanent apparatus of this type were being built for small arms it would no doubt be advantageous to build supports for the lamp and photoelectric cell mounted in a rigid concrete stand in such a manner that the unit could be raised and lowered and moved sideways at right angles to the trajectory, so that the position of the recording area could be adjusted to suit different arms and ammunition. Also it would be advantageous to be able to move the stands clear of the trajectory for test work, and back into place again without having to re-measure the distance between stations. This could be done by mounting the stand on wheels running on rails at right angles to the direction of fire. With the present arrangement the distance between the light beams had to be measured every time the position of the lamps was altered.

VI. Electrical Circuits

Fig. 4 shows the first electrical circuit used with success. The photoelectric cells were all connected in parallel and connected to the input of a resistance capacity coupled amplifier. One stage of amplification was sufficient in this circuit. The output of the 232 tube was connected directly through the Einthoven galvanometer with a balancing circuit to remove the steady component. The amplifications of a current pulse of the type caused by the interruption of the beam of light by the bullet offers a slightly different problem from the amplification of an alternating current. A direct current amplifier would have been ideal but such an amplifier would require an additional adjustable potential in the input circuit. Further, if more than one stage of amplification was used, as was found necessary in the circuit arrangement described later, the number of batteries required would be excessive.

The shape of the current pulse when the bullet interrupts the beam of light probably resembles an inverted V. If the bullet is an inch long and the beam of light an inch thick, the time period of the base of the inverted V

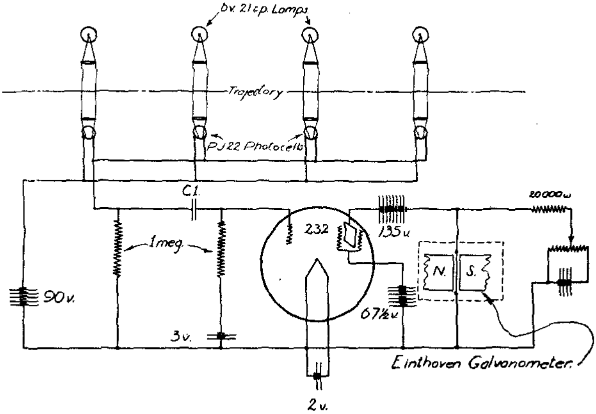

FIG. 4. *Circuit diagram of the single stage amplifier and Einthoven galvanometer.*

would be equal to the time taken for the bullet to travel two inches. For velocities of 2500 ft. per sec. this is $\frac{1}{15,000}$ sec. The apparatus was used with bullets slightly shorter than the above, having velocities as high as 4000 ft. per sec. Here the time of the base of the current pulse would be of the order of $\frac{1}{25,000}$ sec. To obtain a reasonably true record of current pulses of this order, both the amplifier and recording instrument must be made to respond to frequencies much higher than 25,000 per sec. This offers no great difficulty in the case of a resistance capacity coupled amplifier, but does in the case of the Einthoven galvanometer. The fibre supplied with the instrument, according to the makers catalogue, was from 0.002 to 0.005 mm. in diameter and about 65 mm. long; it had a resistance of about 3000 ohms. The natural period of this fibre under the highest practical tension was somewhere between 500 and 1000 cycles per sec. Such a fibre would not follow a current pulse having a period of $\frac{1}{25,000}$ sec. However, if the pulse in the output of the amplifier follows the input pulse reasonably closely the galvanometer fibre should be given a ballistic throw. This throw should have the same shape for each interruption and therefore should produce measurable

results. To make the amplifier as sensitive as possible the resistances in the input circuit should be as high as possible, and to make the response follow the input pulse the condenser C_1 should have a high capacity. The most suitable values for the resistances were found to be 1 megohm as shown, and a capacity of 0.1 mfd. was used for C_1.

In an attempt to make the Einthoven galvanometer respond better to the short period current pulse a special fibre was prepared and put in the instrument. The new fibre was drawn from quartz and was about 25 mm. long and about half the thickness of that supplied with the instrument. One end of the fibre was mounted on a short quartz spring which consisted of a thicker piece of quartz fibre about $\frac{1}{8}$ in. long. The fibre was made conducting by sputtering with platinum. If the movement of the fibre was simply damped harmonic motion one would expect that the natural frequency of the new fibre would be several times higher than that of the heavier fibre supplied with the instrument. Actually under the best conditions the lowest natural period of the new fibre was less than double the old, being of the order of $\frac{1}{1000}$ sec. The control of the fibre, instead of being due to its elastic properties and its supports, seems to be mainly a function of the external circuit and probably the air damping. Curtis (1) by a system of tuned circuits feeding an Einthoven galvanometer, succeeded in making it respond to frequencies up to 10,000 per sec. Probably by means of a further investigation along similar lines a fibre and external circuit could be arranged so that it would respond to frequencies sufficiently high to follow the pulse made when the bullet interrupts the beam of light. Considerably more amplification than was used in the present experiment would be required, as the high frequency combination would not be very sensitive.

Fig. 5 shows three records using this circuit. The upper is a record taken with a .303 service rifle and ammunition giving a muzzle velocity of about 2500 ft. per sec. The lower are records with a high velocity sporting rifle, calibre .280 in., muzzle velocity about 3900 ft. per sec.

The record with the .303 is fairly satisfactory and the distance between the peaks could be measured with a coarse comparator to $\frac{1}{10}$ mm. In the case of the high velocity bullets (lower, Fig. 5) the peaks were not nearly so definite but measurements could be made on their position with reasonable accuracy. Measurements were taken from the top of one peak to the top of the next and then a corresponding distance between two peaks on the 1000 cycle time record directly opposite were measured. The measurement of the tops of the peaks instead of the base was much easier and more accurate. The velocity was calculated from these measurements and the measured distance between the beams of light on the range. From an analysis of the methods of measurement, etc., the maximum errors should not be greater than from 15 to 20 ft. per sec. in the case of the high velocity rifle and about half this in the case of the service rifle. Although the electric disturbance in the photoelectric cell was all over before the fibre reached the peak of its

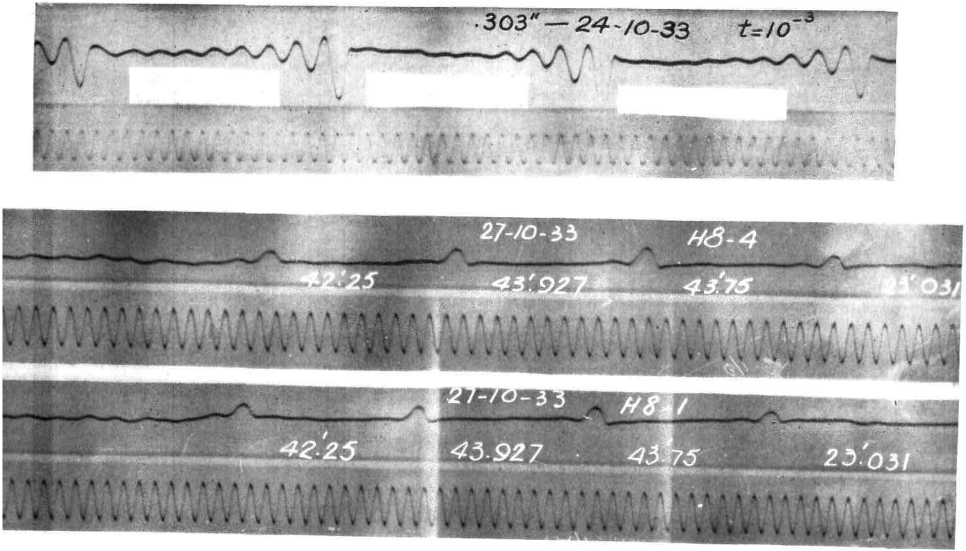

FIG. 5. *Records taken with the single stage amplifier and Einthoven galvanometer.*

swing, the time delay between the disturbance and the peak should be th same for each interruption. Hence the measurement of the top of the peaks should not introduce any appreciable error. Actually greater errors were found. In the case of the high velocity rifle the average error was probably of the order of 50 or 60 ft. per sec. In some cases the records gave the impossible result that the bullet appeared to increase in velocity as it went down the range.

The most reasonable explanation for the magnitude of these errors appears to be that they were due to interference resulting from vibrations of the photoelectric cells and lamps, and to "pick up" from power circuits in the building acting on the transmission lines connecting the photoelectric cells with the amplifier. Vibrations in the lamp stands had been reduced to a minimum by using the stands described in the previous section, but still the records show a background oscillation of the galvanometer fibre between the disturbances caused by the interruption of the beam of light. If the motion of the fibre due to such background oscillations were small compared to the motion of the fibre due to the pulse of current to be observed, they should have a negligible effect on the results. Actually in the case of the high velocity record the background disturbance was often comparable in magnitude to the disturbance caused by the interruption of the beam of light, so the position of the peak to be measured was probably distorted somewhat by interference. This would account for a considerable loss in accuracy.

To overcome this several methods might be adopted. The excursion of the galvanometer fibre due to the pulse of current when the beam of light was interrupted, by no means represents the magnitude of the current pulse in the fibre. On the other hand interference due to vibrations, etc., which are of a frequency lower than the natural period of the fibre will appear on the record without reduction in intensity. If the fibre could be made to respond in detail to the short period current pulse as discussed above, no doubt the effect of interference would be negligible. This was impractical without considerable investigation, so other means of increasing the accuracy were tried.

VII. Thyratron and Einthoven Galvanometer

The thyratron or grid controlled discharge tube offers a convenient relay device for coupling a photoelectric cell to a recording device of the sort required here. For a preliminary test two Western Electric 256A thyratrons were used, in conjunction with photoelectric cell stations Nos. 1 and 2. The thyratrons were connected so that one was controlled directly by each photoelectric cell and the output of the two thyratrons, connected in parallel, was coupled to the Einthoven galvanometer by a resistance and capacity. The thyratrons were connected so that the undesirable disturbances mentioned above were not great enough to start the discharge. The light pulse in the photoelectric cell was made to cause a positive pulse on the grid of the thyratron, and so start the discharge. The discharge was then independent of subsequent fluctuations in grid voltage. Very uniform peaks were obtained

in the Einthoven galvanometer record in this manner. However, to use this method for routine measurement, an amplifier and thyratron would be required at each photoelectric cell station. Without the amplifier the setting of the grid potential of the thyratrons was so delicate that its action was not at all reliable. The arrangement required such a duplication of electrical equipment and batteries that it was not developed to any great extent, particularly since the method described below has some advantages over it.

The circuit arrangement which was found most satisfactory for recording with the Einthoven galvanometer is shown in Fig. 6. In this arrangement

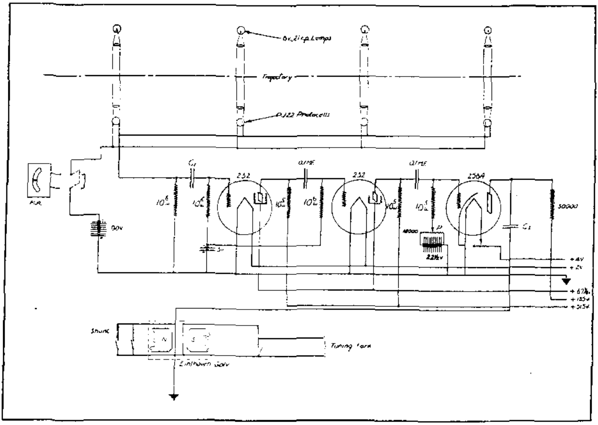

FIG. 6. *Circuit diagram of the two stage amplifier, thyratron and Einthoven galvanometer.*

a two stage amplifier was used of the same type as the single stage unit described previously. The output of this amplifier was coupled to the grid of a 256A thyratron as shown. The plate circuit of the thyratron was connected so that when the positive potential pulse on its grid makes its conducting, it discharges the condenser C_2 (.05 mfd.). Owing to the resistance between the plate and the battery the potential falls low enough to extinguish the discharge. If the grid were set at a more positive potential the circuit would oscillate with the saw tooth wave form used extensively as a time base for cathode ray oscillographs. In the present circuit the grid potential was fixed sufficiently negative that the oscillations in grid potential due to vibrations of the lamps and power "pick-up", etc., were too small to make the discharge start, but the pulse produced when the beam of light was interrupted by the bullet was sufficient to put the tube in a conducting state, thereby discharging the condenser through the galvanometer circuit as

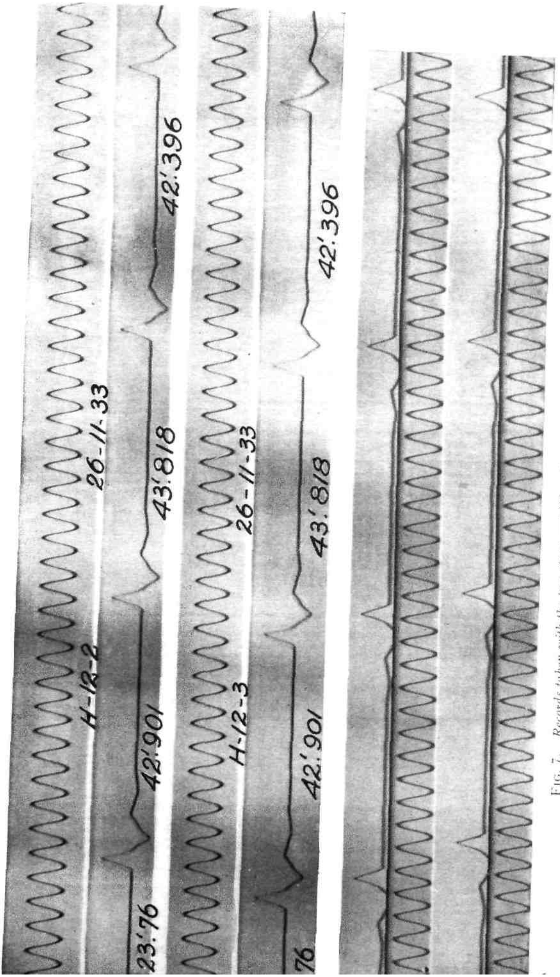

FIG. 7. *Records taken with the two stage amplifier, thyratron, and Einthoven galvanometer.*

shown. An advantage of this circuit is that the galvanometer fibre is affected only by flashing of the thyratron, and small fluctuations due to interference in the input of the amplifier are not recorded. Further, by connecting the galvanometer in the condenser circuit, the current through it reverses suddenly as soon as the discharge stops, so the motion of the fibre is such that a very sharp peak is obtained (see Fig. 7), instead of the rounded top peak as in Fig. 5. These peaks can be measured much more precisely than those in Fig. 5. A further advantage of this circuit is that the current through the galvanometer is always the same and once the proper sensitivity adjustments have been made and the proper shunts chosen the recording part of the apparatus could be used day after day with no readjustments; also it could not be burned out easily by short circuits or loose connections. In the case of the circuit arrangement described previously, where a single stage amplifier was used, there was always a danger of burning out the fibre, as the total plate current was much more than the fibre would stand, and the compensating circuit had to be adjusted carefully with gradually increasing shunts every time the apparatus was put in operation. Any failure of a battery, or a loose connection, would mean the destruction of a fibre. Fig. 7 shows typical records taken with the 256A thyratron and the two stage amplifier. Different optical arrangements were used to bring the two records close together to eliminate any errors due to shrinkage of the film, and for the conservation of space on the film. Two such arrangements are shown.

The possibility of adjusting the electrical constants of the amplifier circuits so that only frequencies corresponding roughly to the pulse being studied were transmitted was also considered. To tune the circuit to one frequency was impracticable as the tuning would have to be changed for each type of bullet. However most of the interference appeared to be of long period, so by adjusting the coupling of the photoelectric cells to the amplifier, low frequency vibrations could be cut out. This was done by varying the size of the condenser C_1. Various sizes from .05 mfd. to .0001 mfd. were tried. The smallest size appeared very satisfactory in that the great majority of the objectional vibrations were eliminated. With this condenser and the lamp currents properly adjusted, the potential of the pulse on the grid of the thyratron appeared to be about 10 to 15 volts above any background noise, so the bias voltage on grid as controlled by potentiometer P, Fig. 6, was not very critical.

The adjustment of the lamps is an important factor in the set-up of the apparatus. The four lamps were fed from one battery with individual rheostats controlling each one. In these rheostats and in all wiring, loose connections must be carefully avoided. Slide wire rheostats might be used if placed away from the influence of vibrations caused by firing. Variable resistances with clamping contacts were used in the present case and were satisfactory. They do not have to be readjusted except when some change is made in the focusing of one of the lamps. The current in the lamps was adjusted to give the correct current in the photoelectric cell as measured by

the microammeter shown in Fig. 6. As it is the quantity of light interrupted by the bullet that is effective in making the record, it is this quantity that was considered in making the adjustments. The current in the lamps was adjusted so that when a rod approximately the diameter of the bullet was inserted in the beam of light, the decrease in photoelectric cell current was the same for each lamp. This decrease was usually about two microamperes. In the case of lamp mountings of Type 1, Fig. 2, this required a total photoelectric cell current of about 9 microamps. Type 2 required about 12, and Type 3 about 5 microamps.

Results and Accuracy

Using four photoelectric cell stations several measurements could be made of the velocity for each round by taking the time between interruptions of the beams of light in any two stations. The result would give the mean velocity between the two stations concerned, or the velocity at a point midway between them. According to ballistic tables the change in retardation over this short range, amounting to less than 1 ft. per sec. change in velocity for the .303 rifle, is much too small to be considered here.

Using the two stations farthest apart, namely, Nos. 1 and 4, the probability of error is least, but by recording the three velocity measurements taken by using the four stations successively in pairs, Nos. 1 and 2, 2 and 3, 3 and 4, an estimate of the accuracy can be obtained. A typical set of results of half a dozen rounds from both rifles used is shown in Table I.

TABLE I

TYPICAL RESULTS

V_{12}, ft./sec.	Diff.	V_{23}, ft./sec.	Diff.	V_{34} ft./sec.
.303 calibre rifle				
2465	28	2437	29	2408
2500	28	2472	28	2444
2462	32	2430	23	2407
2512	27	2485	30	2455
2522	33	2489	24	2465
2477	26	2451	21	2430
Mean diff.	29.0		25.8	
Mean deviation of differences from mean diff.	2.5		2.3	
.280 High velocity rifle				
3858	52	3806	55	3751
3848	39	3809	61	3748
3880	48	3832	49	3783
3842	62	3780	35	3745
3785	32	3753	58	3695
3915	37	3878	43	3835
Mean diff.	45.0		50.2	
Mean deviation of differences from mean diff.	9.0		7.8	

The usual procedure of measuring the film was as follows. If the record was taken on recording paper, the record was measured directly. If the record was taken on a film, a contact print was made and the print measured. The distance between successive peaks of the recording fibre was measured, and the distance between the nearest peaks opposite these on the time scale was used with this and the distance between the beams of light to calculate the velocity.

In measuring the record, the peak of the curve indicating the interruptions of the beam of light was used as the reference point, rather than

its base. The base would represent more accurately the time at which the beam was interrupted, but as the current transient causing the motion of the galvanometer fibre was the same in every peak the error should be negligible. Several records were measured with the base or beginning of the transient as a reference point, as well as the peak, and negligible differences in the results were found. If the measurements gave an absolutely accurate velocity the differences in Column 2 and in Column 4 should be the same for each round, except that those in Column 4 should be lower than those in Column 2 by something less than $\frac{1}{2}$ ft. per sec., as the distances between lamps was such that the second interval was about 0.3 ft. shorter than the first. It is readily seen that the accuracy is not sufficiently great to compare the differences as closely as $\frac{1}{2}$ ft. per sec. The difference in retardation over a range of 43 ft. for a velocity of 3900 ft. per sec. and for a velocity of 3800 ft. per sec. is calculated from approximate ballistic tables to be less than 1 ft. per sec., so neglecting differences in the shape or weight of different bullets, the figures in Columns 2 and 4 should also be practically the same for different rounds if perfect accuracy were attainable. In estimating the probable error from the observed differences, corrections for the above two points were not made as they would have a negligible effect on the results.

The distance between the beams of light was measured to an accuracy of about $\frac{1}{8}$ in. in the 43 ft., so the error due to this should not be greater than about 1 ft. per sec. The distance between peaks on the record could be measured to an accuracy of $\frac{1}{25}$ to $\frac{1}{50}$ mm. The distance between the peaks was usually about 6 cm., so the error in the measurements of the records could not be greater than between 2 and 3 ft. per sec. There did not appear to be any error due to shrinkage of the film or print, as the time record was sufficiently close to the bullet record to make the shrinkage uniform on both.

The tuning fork was compared with a radio-frequency, standard, crystal oscillator which is known to be accurate to better than one part in one million. This standard belongs to the Canadian Radio Commission and a frequency of 1000 cycles per second can be obtained from it. The fork was found to be about 5 cycles per second slow so the velocities indicated in Table I will be too high by about 5 parts in 1000. This error should not enter into this discussion of the accuracy as it will make no difference in comparisons between measurements taken with the same apparatus. In some recent records, not shown, the 1000 cycles from the radio-frequency standard was used directly instead of the fork.

The time lag in vacuum photoelectric cells is approximately the time taken for the electron to travel from the cathode to the anode. This is negligible in the present case. The two stage amplifier was also designed for high frequencies. The ionization time of mercury thyratrons has been measured by Snoddy (2) and found to vary somewhat with the circumstances, but is always less than about six microseconds. The thyratron used (Western Electric 256A) was filled with an inert gas instead of mercury, but the differ-

ence in time delay would not be expected to be appreciable. This lag is neglected in the present discussion, as it should be the same for each interruption.

The largest error is probably introduced by the way in which the bullet enters the beam of light. When the nose of the bullet begins to enter the beam of light the potential on the grid of the thyratron starts to rise. It must rise to a certain point before the tube flashes, thereby recording the position of the bullet. If the total quantity of light interrupted by the bullet was different in different stations, errors might be expected because the bullet would get farther into one beam of light than another before the thyratron flashed. The maximum possible error due to this cause would correspond to the time taken for the bullet to travel its own length. This would correspond to an error in its location of about 1 in. in 43 ft., or an error of approximately 1 part in 500. For the high velocity rifle this means an error of 8 ft. per sec. and for the .303 calibre rifle an error of 5 ft. per sec. If an error of this magnitude occurred as the bullet passed station No. 1 and a second error of opposite sense occurred as the bullet passed station No. 2, the extreme error would be double the above values, or 16 ft. per sec., in the case of the high velocity rifle and 10 ft. per sec. in the case of the .303. As mentioned previously the lamps were always adjusted so that the amount of light interrupted by the bullet would be approximately the same at each station. Any disturbances which came nearly simultaneously with the interruption, such as pickup or vibrations of the cell supports, would influence the above-mentioned error by varying the potential on the thyratron grid but could not make it greater than the figures given.

Summing all errors, the maximum error should not be greater than 19 or 20 ft. per sec. in the case of the high velocity .280 calibre rifle, and about 13 or 14 ft. per sec. in the case of the .303. This applies of course to measurements between any two consecutive photoelectric cell stations. If calculations were made using stations Nos. 1 and 4, the maximum error due to the above-mentioned causes should be about one-third that when the shorter distances between stations are used. In Table I, in the case of the high velocity rifle, no significance is given to the difference between the mean deviations under Columns 2 and 4. A larger table (not shown) of 17 rounds from the same rifle made these figures 6.9 and 8.6 respectively, or a mean deviation from 34 differences of 7.7 ft per sec., indicating a probable error in the difference column of about 6.5 ft. per sec. The maximum error observed appears to be of the same order as that indicated in the above discussion of possible sources of error. In the record of 17 rounds mentioned above, one deviation from the mean difference of 19.5, one of 17.2, three between 14 and 15, and the rest lower, are recorded. Occasional rogue rounds occur when the differences have impossible values. These are thrown out as being due to improper functioning of the apparatus at some point or other, and, more often than not, occurred in experimental trials when the potentiometer *P* (Fig. 6) was set in an unnecessarily sensitive position. In a more permanent

installation with adjustments which are not difficult to make, the number of such rounds should be negligible.

The probable error in taking a velocity measurement on lamps Nos. 1 and 4 should be between 2 and 3 ft. per sec. for velocities of about 4000 ft. per sec. For lower velocities it should be proportionately lower. The accuracy might be improved by using very narrow slits of light, and to a smaller extent by speeding up the camera, so the record would be spread out more. If extreme care were taken in design and precision of adjustment, the probable error might be reduced to about 1 ft. per sec., but probably more satisfactory results would be obtained with a coarser adjustment, and the use of more stations, so that the trajectory could be smoothed out by arithmetic adjustment of the results.

Acknowledgments

The author wishes to thank the Department of National Defence for the loan of two rifles used, and particularly to thank Major G. P. Morrison who took an active part in the investigation and made valuable contributions to its success.

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THE USE OF THE THYRATRON AS A LIGHT SOURCE FOR RECORDING PURPOSES[1]

By D. C. Rose[2]

Abstract

Flashes of light from an FG17 thyratron have been used in conjunction with a rotating drum camera for the recording of time intervals between phenomena. The flashes of light were obtained when a condenser was discharged through the thyratron. Time intervals were obtained on the same record by means of a similar tube controlled by a 1000 cycle alternating voltage from a standard frequency generator. The apparatus has been applied in the present case to the measurement of the velocities of rifle bullets.

The light from a thyratron, when a condenser is discharged through it, has been used by Robertson and Rogers (1) for the stroboscopic measurement of such quantities as phase angle in synchronous machinery. In stroboscopic work the illumination of the moving part concerned is repeated at regular intervals an indefinite number of times. Recently the light from an FG17 thyratron has been used directly to record individual events rather than repetitions of the same event. The thyratron was used as a recording device to improve a method developed by the author for measuring velocities of rifle bullets. The method is described in detail elsewhere (2), but as the use of the thyratron as a recording instrument has many other applications, such as recording for high speed particle counters, or any work where the time interval between events is accurately desired, it seemed advisable to describe its application to the present purpose separately.

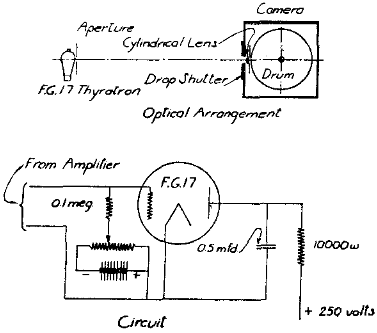

Fig. 1. *Arrangement of the thyratrons and camera.*

The new method of measuring the velocity of a projectile was roughly as follows. The projectile, a rifle bullet in the present case, interrupted beams of light placed along the trajectory. These beams of light were made to shine into photoelectric cells, the current from the cells being recorded. The record indicates the instant the bullet interrupts the beam of light, and by the use of suitable time marks the time interval between interruptions of

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successive beams of light is measured, so the velocity may be calculated. The current pulse in the photoelectric cells, of course, had to be amplified and was recorded by means of an Einthoven galvanometer. The galvanometer has the disadvantage that it is a delicate instrument to use and that the unavoidable inertia of the fibre, though it did not reduce the accuracy of the measurements appreciably, limited the ease with which records could be measured. What was required was a discharge tube which could be flashed without appreciable delay the instant the bullet interrupted the beam of light.

An FG17 thyratron, having a perforated grid gives out a reasonable amount of light, and though not ideally suited for the purpose required, was found to make a good record. The record was taken with the rotating drum camera which had been used in conjunction with the Einthoven galvanometer. The optical system and circuit diagram are shown in Fig. 1. The thyratron was placed about two feet in front of the camera lens with an aperture in front of it about one-half inch wide, so adjusted that it exposed to the camera the area immediately below the opening in the grid. The camera lens was cylindrical, about 15 mm. focus and about 8 mm. wide, being arranged so that it produced an approximate line image of the aperture about 1 cm. long. The circuit was so arranged that each time a positive pulse was impressed on the grid the tube discharged the condenser. The resistance in the anode circuit caused the voltage to drop, thereby stopping the discharge in the usual manner with such circuits.

For a time record another thyratron was used, placed beside the first and connected in a similar manner, except that the grid was excited by a 1000 cycle voltage from a standard frequency generator. The tube would not oscillate in a satisfactory manner at a frequency of 1000 cycles per sec., but worked very well at 500 cycles with the 1000 cycle excitation.

Records are shown in Fig. 2. The stronger lines represent the flashing of the first thyratron when the bullet interrupted successively four beams of light. The weaker lines represent the flashing of the time recording tube. Snoddy (3) has measured the ionization time of mercury thyratrons and found it to vary somewhat with conditions but to be less than about six microseconds. It would be expected to be the same for each pulse, so in measuring time intervals the error due to lag in the tube should be

considerably less than this. Such an error was negligible in the projectile velocity measurements. The time taken for the discharge of the condenser was not measured but, judging from the nature of the records, it was not longer than a few microseconds. The width of the line did not appear to be appreciably greater when the camera drum was stopped than when rotating at a film speed of about 500 cm. per sec. The amount of light obtained with the FG17 and the present optical system was just sufficient to obtain a good record on verichrome film. Further developments of this method of recording are being undertaken.

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REVIEWS AND NOTES

The Thermal Decomposition of Gaseous Methyl Iodide

With the exception of one qualitative investigation by Kahan (1) no work has been done on the thermal decomposition of gaseous methyl iodide. It seemed worth while investigating this reaction, since by analogy with the decomposition of hydrogen iodide it might proceed smoothly to ethane and iodine, or else to ethylene, hydrogen, and iodine.

Twenty-two runs were made under various conditions and the following results were obtained.

With an empty Pyrex bulb the reaction proceeds at a measurable rate above 450° C. The pressure increase accompanying the reaction is from 17 to 19%. At 510° C. and an initial pressure of 31 cm. the time for a 10% increase in pressure was 2 min.; for 15%, 8 min.; and completion was reached in about 45 min. A large amount of carbon is deposited, and iodine is formed during the reaction.

In a bulb packed with powdered Pyrex glass there was a pressure *decrease* of about 10% above 450° C. Runs at 435 and 400° C. showed increases up to 10%, followed by a decrease.

From the point of view of kinetics the reaction is obviously too complicated to be of much value. The investigation was therefore abandoned, but it was thought that the results might be worth noting.

We wish to express our indebtedness to the National Research Council of Canada for a fellowship awarded to one of us (R. D. McD.), during the tenure of which this work was performed.

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An Easy Method of Removing Adhering Rubber Tubing and Stoppers from Laboratory Glassware

In laboratory work it is very often necessary to take apart connections between sections of glass apparatus that has stood for a long period. Trouble arises in most instances in connection with the removal of rubber tubing or stoppers from glass tubes and flasks, in many cases necessitating cutting off the adhering rubber.

A simple and useful method for the removal of adhering rubber tubing or stoppers from laboratory glassware was devised. The procedure is as follows: the glass tubing, or any other glassware from which the rubber tubing or stopper is to be removed, is connected in any convenient manner with a steam outlet (or a supply of very hot water) in such a way that the steam will circulate inside the glassware. The steam heats the glass and softens the adhering layer of rubber in about two minutes. The rubber tubing or stopper may then be removed by gentle working.

This procedure has been tried out on a variety of glassware and has been found to be very satisfactory. Rubber–glass connections which had been previously subjected to very high temperatures did not separate as easily and cleanly, but, nevertheless, this treatment permitted the removal of the rubber much more readily than otherwise.

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THE BEECH BARK DISEASE

A *NECTRIA* DISEASE OF *FAGUS*, FOLLOWING *CRYPTOCOCCUS FAGI* (BAER.)[1]

BY JOHN EHRLICH[2]

Abstract

This paper is concerned with a disease of beech (*Fagus*) caused by the sequent activity of a scale insect and a parasitic ascomycetous fungus. Although the writer's studies have dealt almost exclusively with the American beech (*F. grandifolia* Ehrh.), the insect has been observed on *F. grandifolia* var. *caroliniana* Fern. and Rehd., as well as on the European beech (*F. sylvatica* L.) and a number of its varieties. The name "beech bark disease" is proposed.

The European history of this disease, gleaned largely from the literature on the insect, is reviewed, beginning with the first reports of injury at the middle of the last century in England and Germany, tracing its reported activity to the present, and the conclusion is reached that the disease is not now regarded as so serious an affliction of beech as formerly and that it is widespread and enphytotic in central and western Europe. In America the disease has been active for approximately 15 years; it first attracted attention in the vicinity of Halifax, Nova Scotia, and has spread steadily until it is now active throughout Nova Scotia, in southern New Brunswick, and in localized areas of eastern and south central Maine, although the insect pathogen has been present also for perhaps a decade in eastern Massachusetts. A pathological line-plot cruise of typical affected areas in Nova Scotia and New Brunswick demonstrated that, in the stands examined, the beech infected by the fungus had attained a uniform mean of 90% and that in the regions infested for a longer period approximately 50% of the beech had been killed at the time of observation. The conclusion is reached that the disease in America is assuming the proportions of a fatal and spreading epiphytotic.

The first indications of the disease are the appearance of the minute insect on the bark of trunk and branches, and the subsequent development of fruiting bodies of the fungus in the areas previously occupied by the insect. The insect probes the living tissues of the outer bark, extracting protoplasmic materials and causing the death of punctured cells. The shrinkage of groups of killed cells leads to tearing of the periderm, which enables the fungus to initiate infection. Once past the barrier of phellem, the fungus is able to advance through

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the living tissues of bark, cortex, phloem, cambium, and sapwood. Death of the infected tissues interferes with normal conduction and storage in the trunk and results in a progressive killing of the tree, which becomes complete when the fungal lesions have coalesced sufficiently to effect an interruption in conduction. As infestation progresses, the foliage and twigs dry and die, whole branches cease to leaf out, and large areas of bark on the trunk crack, usually loosen from the wood, and eventually fall away. On younger trees infection is less abundant, the fungus advances less readily, and individual lesions frequently become surrounded by healthy bark and wood, and eventually form depressed areas but never typical scalariform cankers.

The insect (*Cryptococcus fagi* [Baerensprung]) which initiates this disease has been reported from Germany, Switzerland, Czechoslovakia, France, Belgium, Holland, Sweden, the British Isles, eastern Canada (where it was first reported in 1914 and has probably been present since 1890), and the northeastern United States (where it was discovered by the present writer in 1929). Synonymy, previous descriptions of the insect (a member of the Homopterous family Coccidae), and its life history are reviewed, with personal notes on habits, dissemination and long range distribution, and factors influencing infestation.

The fungus following the insect in America is a species of *Nectria* in the *coccinea* group as defined by Wollenweber. Its specific identification must await a thorough comparative study of the American forms, but spore measurements indicate that it is an undescribed variety of *Nectria coccinea* (Pers.) Fries. A brief description is given. The fungus has been repeatedly isolated and grown in pure culture on a variety of media, producing conidia and ascospores. The influence of low temperature, aeration, and hydrogen ion concentration are discussed and data given on the height of ascospore discharge. Spore-trap experiments demonstrated that ascospore discharge follows rains of sufficient duration to wet the bark and continues until after the surface of bark and perithecia appears dry; both ascospores and macroconidia are air borne.

The role of each of the pathogens in producing the disease has been demonstrated. The evidence consists of the following: (1) the constant association of the organisms with the disease, (2) absence of the disease when one only is present, (3) consistent isolation of the fungus from infected tissues, and (4) the inability of the fungus to infect tissues not infested by the insect and its ability to infect those infested. The insect probes the bark, resulting in a rupture of the periderm attendant on death and shrinkage of the living cells of the bark on which the insect feeds. This enables initial infection by the fungus. The fungus grows parasitically, causing the death and destruction of the storage and vascular systems of the trunk and branches.

The fungus is shown by experiments to be able in general to enter and infect extensively only bark on which the insect has been present for approximately a year or longer. Observations in each of three consecutive years on tagged trees in permanent sample plots show that, once the trees become generally infested by *Cryptococcus fagi*, *Nectria* infection develops on the majority within three years and kills some of them in one or two years. Later, almost the entire stand becomes infected and the amount of infection builds up rapidly, with resulting mortality.

A pathological line-plot cruise of 200 plots containing 4,483 beech shows on analysis small but definite positive correlation between percentage of beech infected by *Nectria*, and percentage of beech in stand, position on ridge, and steepness of slope; severity of infection shows definite correlation with diameter (d.b.h.),* and questionable correlation with forest type, and crown density. Mortality, expressed as percentage of beech (over 3 in. d.b.h.) killed, is correlated with diameter, crown class, and position on ridge, and questionably with forest type, and steepness of slope.

The disease can be controlled on ornamental trees by early eradication of the insect with insecticides. Control in forest stands should aim first at the salvage of infected, dying, and recently killed timber, for the purposes of obtaining the greatest possible return on a rapidly depreciating investment and of preventing the development in the slash of unsanitary conditions conducive to further deterioration in the remaining stand. Second, control should aim at the possibility of combatting the insect pathogen with fungal and insect enemies. Third, it should, by forest management, favor beech on broad ridge tops rather than steep slopes, and cull the larger trees in an attempt to produce changes in the environment designed to restrict activity of the pathogens and substitute a younger, less susceptible stand.

*Diameter at breast height.

I. Introduction

The subject of the investigation reported in the following pages is a disease of beech *(Fagus)*, new to America. Its rapid and destructive spread has created the task of determining its cause and its *modus operandi*, and from the knowledge obtained with respect to these features the obligation of endeavoring to formulate a technically sound and economically feasible program of control. Underlying problems in both directions, as well as related ones, have been investigated and are discussed in this paper.

Associated with the disease are the attacks of an insect, a minute coccid conspicuous by reason of its woolly, white, wax secretion. This has been the topic of many entomological studies and observations, especially in Europe. The present research has disclosed the fact, however, that the insect merely paves the way for a fungus which is responsible for most of the injury and ultimate death of the beech. This fact has necessitated a change of viewpoint; the emphasis is here directed to a disease of a forest and shade tree, rather than to an insect pest or fungi associated with it.

II. Suscepts

The disease is known on the two western species of beech only: the European *(Fagus sylvatica* L.) and the American *(Fagus grandifolia* Ehrh.). Although the disease is common on these two species, certain of their varieties are infested to a greater or less extent by the insect. Thus in Great Britain the varieties with copper-colored or bronze-colored leaves *(F. sylvatica* var. *atropunicea* West., etc.) are reported as more or less attacked by the insect (Gillanders 1908, Green 1915, Forestry Commission 1926) and the weeping beech (probably *F. sylvatica* var. *pendula* Loud.) as relatively resistant (Newstead 1901, Carpenter 1903). In Holland Schenk (1924) described a "bruin" beech scion *(F. sylvatica* var. *atropunicea* West.) as only sparsely infested, while the common beech stock below the graft union harbored a dense mass of the insects. In the Arnold Arboretum, Boston, Massachusetts, the writer has observed light natural infestation by the insect on the dwarfed contorted *F. sylvatica* var. *tortuosa* Pépin and on the southern variety of the American beech *(F. grandifolia* var. *caroliniana* Fern. and Rehd.). This enumeration is in no sense complete since neither in Europe nor in America have artificial infestation experiments been performed.

III. Names

Most of the early observations were made by entomologists and horticulturists who were not accustomed to go beyond the injury caused by an insect or fungal pathogen. Accordingly few names have been applied to the disease itself. The only European designation known to the writer is Rhumbler's (1914a, b, 1915, 1922), "Schleimfluss-Wollaus-Nectria Krankheit." In America the first references to the disease were made by Faull (1930 a, b) who pointed out the importance in Nova Scotia of the "bark disease of beech."

In preliminary accounts of investigations the writer referred to it as the "bark disease of the beech" (Ehrlich 1931) and as the "beech bark disease" (Ehrlich 1932b). The name "beech bark disease" is used in this paper.

IV. History and Range

IN EUROPE

The early history of the disease is necessarily largely the history of references to the insect (*Cryptococcus fagi* [Baer.]) which is its initial cause. A detailed account of this insect is given below in Section VII A. As early as 1849, however, M'Intosh referred to the outbreak as a disease and noted that it had affected the beech for some years. Since 1849 accounts of injury and mortality have appeared in many entomological papers. The only detailed studies of the effects following insect attack and associated fungi are by Hartig (1880), Boodle and Dallimore (1911), and Rhumbler (1914 a, b, 1915, 1922, 1931*). Hartig described the effects of insect feeding on the tissues of bark, phloem, and cambium, and concluded that fungi which follow, such as *Agaricus adiposus* Fr. and *Nectria ditissima* Tul., are entirely secondary and of little importance. Boodle and Dallimore drew the following conclusion from their observations: "While the Coccus is doing very little harm, certain fungoid pests account for a serious amount of injury, which is usually credited to Coccus. On all the dead trees pointed out to us as having been killed by Coccus, we found destructive parasitic fungi present, which alone would account for the death of the trees." (Boodle and Dallimore 1911: p. 341.) The fungi mentioned are *Nectria ditissima* Tul. on the bark of the trunk, and *Melogramma spiniferum* De Not. on the bark of the roots at the base of the trunk. Rhumbler (1914 a, b, 1915) concluded from extended study and observations that the insect alone never causes the death of a beech, but hastens the effects of a preceding slime-flux infection and the subsequent activity of *Nectria ditissima* Tul. Later (Rhumbler 1922) he described three types of slime-flux infection and concluded his observations on the etiology and course of the "Schleimfluss-Wollaus-Nectria" disease by assuming that an unknown agent enables the development of primary slime-flux lesions (S_I, S_{II}) and light infestation by *Cryptococcus fagi* (Baer.); these three agents are held to be mutually helpful and to give rise to the dangerous S_{III} stage which results in killing, aided by other fungi (especially *Polyporus* [*Fomes*] *fomentarius* [Fries] L. ex. Gill. and *Nectria ditissima* Tul.) and by the borers *Lymexylon dermestoides* L. and *Xyloterus domesticus* L.

The absence of the pathological viewpoint in most of the other European accounts makes it hazardous to evaluate the extent and character of the disease abroad; but one cannot escape the conclusion that it is widespread in Europe, following in general, but certainly not completely, the range of *Cryptococcus fagi* (see section VII A below), and that the disease is enphytotic and occasional rather than epiphytotic in nature.

In North America

In America the situation has been quite the reverse. The first recorded outbreak occurred in Nova Scotia about 1920, some 30 years after the first known appearance of the insect on ornamental European beech in the Halifax Public Gardens, and some five years after its recognition as general in beech stands in the vicinity of Halifax.* Graham (1927) stated that lumber companies operating along the south shore of Nova Scotia reported during the winter of 1925-26 that the beech stands in that part of the province were in danger of being entirely destroyed by this disease. The first published references to the disease were by Faull (1930a, b) who observed its effects in various parts of the province. Countless verbal statements to the writer as well as personal observations beginning in 1930 have made it clear that death, which is now known to result from infection of the insect-infested trees by a species of *Nectria*, has followed *Cryptococcus fagi* in its steady march in all possible directions from Halifax. By 1930 the disease had become so firmly established throughout the Nova Scotia mainland that hardly a beech stand could be found in which there had not already been some mortality, and in the island of Cape Breton symptoms were becoming evident everywhere. The writer has not visited Prince Edward Island and is therefore uncertain whether the fungus has attacked the insect-infested beech in that province; the Dominion Plant Pathologist, stationed at Charlottetown, writes that he has not yet observed infection. Meanwhile in New Brunswick infection could be found in 1930 as far from the interprovincial boundary as the town of Albert and much mortality had already taken place in Westmoreland County. The spread of infection during the next two years was observed by the writer both in Cape Breton and in New Brunswick: by 1932 the disease had already begun to take its toll in Cape Breton and had advanced in New Brunswick at least 20 miles along the shore of the Bay of Fundy, passing the village of Alma in Albert County. Meanwhile, further scouting showed that the insect had already become established at various points over a much larger area involving the whole of southern New Brunswick (including the counties of Westmoreland, Albert, Saint John, Kings, Queens, and Charlotte) and that young spot infections by the *Nectria* already existed in certain of the insect-infested stands.

The proximity and continued encroachment of this veritable epiphytotic in the Maritime Provinces have stimulated a keen lookout in New England, since neither of the pathogens was known to occur south of the international boundary. As early as 1929 infestations of beech were discovered by the writer in eastern Massachusetts (Essex, Middlesex, Suffolk, and Norfolk

**Thus, two woodsmen, George Dickey, chief district forest ranger, Musquodoboit, Halifax Co., N.S.; George Horne, sub-ranger, Enfield, Halifax Co., N.S., well acquainted with the outlying parts of Halifax County, told the writer that beech had begun to die in the Musquodoboit Valley before 1920, and in the vicinity of Enfield by 1923. A lumberman now operating in Yarmouth County stated in a letter and later told the present writer that, "In 1920, after I returned from the war I had a man clear up quite a few beech trees at my home in Halifax County. These trees had died from some cause . . . . I now feel this was the first of the disease that I noticed. The same year the beech in Colchester County began to die and in 1921 I noticed about an acre near Carleton, Yarmouth Co., that was about all dead." (Boutilier, 1929.)*

Counties) and in 1931 members of the United States Bureau of Entomology (Melrose Highlands Laboratory) found the insect in south central Maine (Ehrlich 1932a). Further surveys in Maine by the State Entomologist during 1932 led to the finding of additional insect outbreaks at many points in an area in the south central part of the state involving parts of the counties of Waldo, Lincoln, and Knox, and an older infestation in Washington County (eastern Maine) adjoining the southern New Brunswick infestation in Charlotte County. A careful watch has been kept by the writer for possible *Nectria* infection and the development of the disease in eastern Massachusetts, but as yet none has been discovered. In the autumn of 1932, however, the State Entomologist for the State of Maine informed the present writer that he had just discovered what appeared to be infection by *Nectria* on insect-infested beech in one of the south central Maine localities, and that similar infection had been found more widespread, more advanced, and associated with mortality in the Washington County areas. In November, 1932, the writer visited the infected stand at Liberty (Waldo County, Maine) and determined that both fungus and disease were the same as in the Maritimes (Ehrlich 1933a, b). The known range of the insect and of the fungus associated with the disease have been indicated on a sketch map (Fig. 1). It will be observed that both are still limited to the maritime margin of the continent; whether this is due merely to the chance origin of the infestation by the sea or to ecological factors which may limit westward distribution can not yet be asserted.

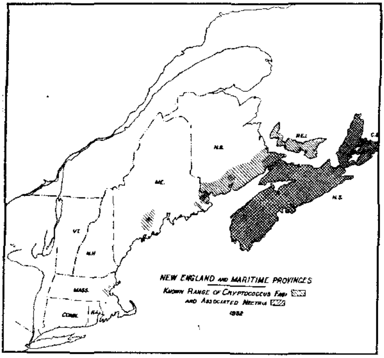

FIG. 1. *Known range (1932) of the pathogens of the beech bark disease in the Maritime Provinces and New England.*

Summarizing, the European accounts indicate that pathological complications of attack by *Cryptococcus fagi* are occasional and become serious only in localized areas. In eastern North America on the contrary, a distinctly epiphytotic disease has developed; a large reserve of inoculum has been built up and fungal infection follows in most cases so closely and abundantly on insect attack that death comes quickly. Furthermore, the pathogens are distributed so rapidly that their progress as well as that of the disease can be observed from year to year over large areas. The disease in North America is known to be active in Nova Scotia and in portions of New Brunswick and Maine.

V. Importance

HISTORICAL RÉSUMÉ

In Germany, Hartig (1880) reported occasional killing of infested trees and concluded that considerable damage might result in young stands, especially those on poor soils, but that in older stands the disease became injurious only in individual cases. Altum (1881) reported that much injury was being caused but gave no data. Nüsslin (1905) merely stated that the importance to forestry of the disease following the woolly beech scale was not slight. Bertelsmann (1913a) gave figures for the district of Ziegelroda, in which, 10 years after an infestation had first been observed, the loss in beech killed amounted to approximately 4000 cubic metres in one year and in each of the following two years, 3000; he pointed out the importance of salvaging such timber promptly, since the wood decays—once the tree is dead—in an incredibly short time. Rhumbler, who published extensive observations on the course of the disease, reported that from 15 to 20% of the heavily infested trees are killed (1931). Thus it may be reasonably concluded that in Germany the disease, though common, is not epiphytotic nor of outstanding economic importance.

In Holland, injury is regarded as less important than was thought at one time (Anon. 1921). Although it "is not taken seriously by the Belgian foresters" (Anon. 1910 : p. 643), Vayssière stated in 1926 (p. 350) : "J'ai pu constater son importance économique en Belgique où, certaines années, les Hêtres de la forêt de Soigne ont leur tronc entièrement couvert par la sécrétion blanche du *Cryptococcus fagi* qui leur donne un aspect tout à fait caractéristique."

In England, with the realization that a new pest was at hand, much fear was experienced as to its possible injurious effects but no actual estimates of damage were made. In later years, either the insect became less injurious or else earlier fears proved to be ungrounded. Thus as early as 1851, C.C. stated in a horticultural journal that "about six or seven years ago the ornamental Beeches, in many of the gentlemen's grounds in Yorkshire were thus attacked and gradually destroyed . . . . " (C.C. 1850 : p. 11). Foggo (1863) wrote of the "fatal disease of ornamental beech." In 1898 Miss Ormerod quoted a letter from Lord Burton, who referred to " . . . . the infestation which is destroying all the Beech trees in the woods here" (Ormerod 1898 : p. 6); two years later she spoke of the insect associated with the disease as very destructive (Ormerod 1900). Newstead, who is responsible for the best entomological account, reported that in eastern Surrey many of the finest trees were being destroyed (1900); that the insect is one of the most destructive of native coccids (1903); and that it is one of the most destructive pests of beech (1905). Theobald (1905 : p. 137) referred to it as "the most serious forest tree pest we have." Perhaps more important than economic losses is the effect which this unfortunate situation was capable of producing in the Rev. Wilks: "We fear the Beech is doomed all over the country, and that the next generation will only know by pictures and reports how gloriously beautiful

our forest Beeches have been" (Wilks 1901-02: p. 599). In 1907 the insect was characterized as "one of the most destructive pests against which the arboriculturist has to contend" (Boulger 1907: p. 276). In 1908 MacDougall reported that *Cryptococcus fagi* continues its destructive work. The same year Webster stated: "It is only of late years that the terrible ravages have been felt severely, and that owners of woodlands have been driven to do everything in their power to combat its injurious effects" (Webster 1908 : p. 257). Gillanders (1908 : p. 234) characterized the insect as "one of the most dreaded pests of the arboriculturist." It might be judged from the foregoing references that the insect and its disease were indeed major calamities in the England of three decades ago. But in 1911 Boodle and Dallimore made a painstaking effort to discover just what the effects of *Cryptococcus fagi* really were and how serious they might be; their conclusions are worthy of note: "That the Coccus has not assumed an epidemic state is apparent everywhere" (Boodle and Dallimore 1911 : p. 341). "That it is doing any serious amount of harm, or that it is likely to do so, is doubtful. In some of the early records the disease appears to have been quite as plentiful as it has been of late years, and to have caused the same uneasiness, but the worst expectations were never realized." (*Ibid:* p. 340.) Apparently this conclusion was justified, for as recently as 1925 we read: "The view that Beech Coccus is a serious enemy of the beech is a prevalent one . . . . That view has no support in fact" (Munro 1925 : p. 284). The following year the same view was expressed by the Forestry Commission (1926). But Boodle and Dallimore added the following, which was repeated in 1925 by Munro: "While the Coccus is doing very little harm, certain fungoid pests account for a serious amount of injury, which is usually credited to Coccus. On all the dead trees pointed out to us as having been killed by Coccus, we found destructive parasitic fungi present, which alone would account for the death of trees. The same fungi kill or injure other kinds of trees on which the Beech Coccus does not occur." (*Ibid* : p. 341.) The two most common parasitic species were stated to be *Nectria ditissima* Tul. and *Melogramma spiniferum* De Not.*

While the enphytotic nature of the disease following *Cryptococcus* attacks in Europe has been reflected in only minor economic importance, in America the recent epiphytotic attack is producing losses of great magnitude and even greater potential importance. As early as 1914 in the first published account of the discovery of the insect here, Braucher warned that "if it once becomes firmly established in our beech woods and thrives as it promises to do, it is impossible to foresee the damage that it may cause" (Braucher 1914 : p. 15). By 1926 Hutchings wrote: "Throughout the central and southern parts of Nova Scotia the trees are reported to be in a very serious condition, and there is considerable apprehension as to the losses it has already brought

**Personal observations and consultations with forest entomologists and pathologists in England during the autumn of 1933 have convinced the writer that Cryptococcus fagi is widespread, that parasitic fungi do not here ordinarily follow infestation by the insect, and that no damage can be attributed to the woolly beech scale or to associated fungi in England. The fungi mentioned by Boodle and Dallimore (1911) have been encountered repeatedly, but only on logs, stumps, and standing trunks which had died from other causes.—Author, Kew, March 1934.*

about, the gradual spreading of the trouble to fresh areas being a source of further anxiety . . . . The trees eventually succumb to the attack." (Hutchings 1926 : p. 116.) In the same year the Minister of Agriculture for Canada reported that "the beeches are dying in considerable numbers throughout the infested area" (Motherwell 1926 : p. 98). When in 1929 Faull made a pathological reconnaissance of the forests of Nova Scotia he was at once impressed by the fact that a virulent disease of fungal origin was active in the insect-infested beech, and decided that this "bark disease of beech" was perhaps one of the two outstanding diseases of hardwoods in the province (Faull 1930a, b). At the same time the Associate Dominion Entomologist in charge of the Division of Forest Insects reported that *Cryptococcus*, associated with certain fungi which apparently follow in its wake, was the cause of very extensive injury (Swaine 1930 : p. 45). As a result of observations throughout Nova Scotia during the summers of 1930 and 1931, the present writer stated: "There is hardly a stand of maturing beech in the province in which the disease can not be found, and in most of them a large portion of the trees are either dying or already dead" (Ehrlich 1932b : p. 43). During the past year the Chief Forester of the province judged that of the volume of standing beech timber, estimated at 304,661,000 cu. ft., the disease had already destroyed approximately 100,000,000 cu. ft., or slightly less than one-third (Schierbeck 1932 : p. 17).

In Eastern Canada

The extensive mortality resulting from this disease and the probability of its continued spread prompted the writer to attempt an evaluation of the actual damage resulting. It was realized that such data might also be of value in securing statistical substantiation of theoretical explanations of the influence of environmental factors on the incidence of infection and mortality, which might form the basis for possible silvicultural control experiments. It was therefore decided to employ a modification of the foresters' line-plot cruise, recording data not only on diameter and crown classes for each species and on pathological conditions for beech, but also on factors possibly influencing the disease, such as forest type, percentage of beech in stand, crown density, aspect, position on slope, percentage of slope, and proximity to sea water.

Five regions were selected, each in a different county (Fig. 2); three of them were in portions of the Nova Scotia mainland known to have been attacked for at least 10 years, and in which it might accordingly be assumed that a certain equilibrium had been reached between the aggressiveness of the pathogens and the limiting influence of an environment changed by the progress and effects of the disease. The other two areas, one in Cape Breton and one in New Brunswick, were selected in regions known to have been attacked within approximately the last four to six years, and in which the damage had not yet attained its maximum, but in which possible differences in the early course of injury could be analyzed. The general procedure in making this survey was the standard one employed in timber-estimating

by the line-plot method. In each region typical beech ridges were selected and laid out in such a manner that a fair sampling of approximately 0.5% of the area could be taken; this was usually done by running parallel compass-courses over the area, taking 0.1-acre sample-plots every 10 chains, offsetting at the end of a course for 20 chains (0.25 mile), and then working back on a new line parallel to the old. The compass man (a trained forester) took

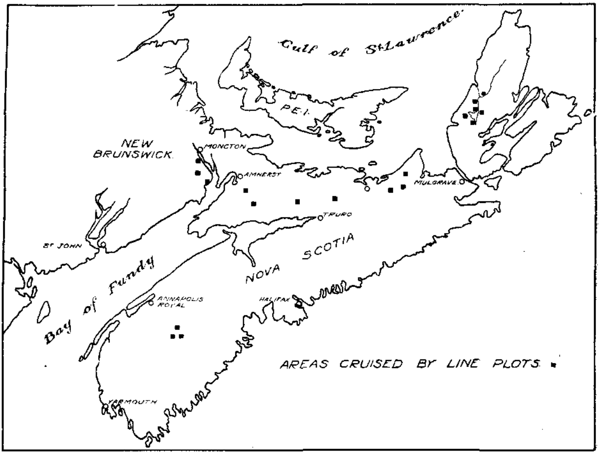

FIG. 2. *Location of areas in Nova Scotia and New Brunswick cruised by line plots.*

preliminary general notes on the plot while the observer ran a measured string around the plot, half a chain to one side of the two-chain tape, two chains parallel to the tape, and half a chain back to the tape, enclosing a plot of one square chain (0.1 acre). The writer made all observations and the compass man recorded the notes on a specially designed form sheet. Each tree was chalked as observed to eliminate the possibility of duplication or omission. The general data on this survey are presented in Table I. It will be observed from the table that a total area of approximately 6.25 square miles (4000 acres) was cruised, in which 200 plots of 0.1 acre each were examined, totalling 20 acres of beech stand in which every tree over three inches in diameter at breast height (4.5 feet) was examined. The total number of trees of all species was 8160, of which 4483 were beech. The mean number of trees of all species per plot was 40.8; of beech, 22.4.

The composition of all line plots by species and diameter classes is shown in Table II. In addition the actual number of beech in each diameter class

TABLE I

GENERAL DATA ON LINE-PLOT SURVEY, 1932

	Nova Scotia mainland	Cape Breton and New Brunswick	Total
Approximate area cruised (in acres)	1,400	2,600	4,000
Approximate percentage of cruised areas examined	0.5	0.5	0.5
Size of individual plots (in acres)	0.1	0.1	0.1
Acreage of all plots	7.0	13.0	20.0
Number of plots examined	70	130	200
Total number of trees (over 3 in. d.b.h.)	2,802	5,358	8,160
Total number of beech (over 3 in. d.b.h.)	1,497	2,986	4,483
Mean number of trees per plot	40.0	41.2	40.8
Mean number of beech per plot	21.4	23.0	22.4

TABLE II

COMPOSITION OF ALL LINE-PLOTS, BY SPECIES AND DIAMETER CLASSES

Genus and species	Per cent of each species in each diameter class						Species totals	
	4–7 in.	8–11 in.	12–15 in.	16–19 in.	20–23 in.	24–27 in.	Number of trees	Per cent of total number of trees
Number of *Fagus* trees	2,506	1,337	519	109	12	—	4,483	—
Fagus grandifolia	55.9	29.8	11.6	2.4	0.3	—	4,483	54.9
Acer saccharum and *A. rubrum*	58.8	25.9	11.0	3.0	0.8	0.4	1,351	16.6
Betula lutea	41.4	28.7	16.9	7.2	5.3	0.5	947	11.6
Abies balsamea	83.7	13.6	2.6	0.1	—	—	821	10.1
Picea rubra and *P. glauca*	65.7	20.4	9.4	4.5	—	—	245	3.0
Tsuga canadensis	56.4	21.8	18.2	3.6	—	—	55	0.7
Betula papyr fera	49.2	30.7	16.6	1.5	2.0	—	199	2.4
Populus grandidentata	38.1	61.9	—	—	—	—	21	0.3
Carpinus caroliniana	100.0	—	—	—	—	—	17	0.2
Quercus rubra	43.8	37.5	18.7	—	—	—	16	0.2
Pinus strobus and *P. resinosa*	40.0	20.0	20.0	20.0	—	—	5	0.1
					Total		8,160	100.1

is given. The table shows that for the plots as a whole the hardwoods, beech (*Fagus grandifolia* Ehrh.), maple (*Acer saccharum* Marsh., *A. rubrum* L.), and yellow birch (*Betula lutea* Michx.), constituted 83.1%, of which 54.9% was beech. The common softwoods constituted only 13.8%, of which fir (*Abies balsamea* Mill.) far outranked spruce (*Picea rubra* Link, *P. glauca* Voss) and hemlock (*Tsuga canadensis* Carr.). The remaining 3.2% consisted of other species.

The most obvious measure of the importance and activity of the disease is the abundance of the pathogens. One measure of abundance is the percentage of the susceptible trees infested by *Cryptococcus* and infected by *Nectria*. Preliminary observations gave convincing evidence that in a diseased stand practically every beech harbors a certain number of the insects, and that, although the rate of increase of the infestation varies, the number ultimately decreases to zero as fungal infection destroys the tissues on which the insect is dependent for food, to the point of killing the tree, so that neither the percentage of beech infested nor the amount of infestation at a given time of observation was judged capable of yielding significant data. Evidence of *Nectria* infection, on the other hand, remains even after death and was judged to afford a more reliable index to the amount of disease which was or had been present.

The percentage of beech in each plot which showed symptoms or signs of *Nectria* infection and the mean or plot percentages for each county were secured. Table III shows the number of sample plots, the total number of

TABLE III

PERCENTAGE OF BEECH INFECTED BY *Nectria* (PLOT-PERCENTAGE MEANS), BY COUNTIES

Regions	Nova Scotia mainland			Cape Breton and New Brunswick		All counties
Counties	Annapolis	Cumberland	Antigonish	Inverness	Albert	
Number of sample plots	29	24	17	100	30	200
Total number of beech in all plots	691	467	339	2,247	739	4,483
Mean plot per cent of beech infected	**92.7** ±1.8*	**87.5** ±1.7	**86.5** ±3.3	**89.4** ±1.4	**90.9** ±1.3	**89.6** ±0.77
Probability odds**	>100 to 1	>100 to 1	>100 to 1	Very great	Much > 100 to 1	Very great

**The figure following the plus-or-minus sign (±) is the standard error of the mean, computed from the individual plot percentages by way of the standard deviation, according to the formula:* $\sigma_m = \frac{\sigma_x}{\sqrt{n}}$, *where* σ_m *is the standard error,* σ_x *is the standard deviation, and* n *is the number of sample plots.*

***Taking the mean percentage of trees infected as 90%, the values in this row were obtained by computing* χ^2 *from the actual numbers of trees infected in each plot and the numbers expected with a mean percentage infected of 90. The formula employed is:* $\chi^2 = \Sigma\left(\frac{x^2}{m}\right)$, *where* x *is the difference between the actual and expected number of trees infected, and* m *is the expected number.*† *Where* n *(one less than the number of sample plots) was 30 or less, the value of P was read by entering the table of* χ^2 *in Fisher (1930). These probabilities have here been converted into odds. Where* n *was more than 30, the formula* $\sqrt{2\chi^2} - \sqrt{2n-1}$ *was employed; when the first term of the formula was less than the second, P was judged to be greater than 0.5.*

The values in this row measure the probability that a sampling (identical with the present one in number of plots and number of beech in each plot), made from a population in which trees were infected at random with a constant probability of infection for each tree (this probability being the 90% actually found), would show deviations as great as, or greater than, those found in the present sampling.

†*See footnote on p. 605.*

beech in all plots, and the mean percentage of beech infected in each county and in all counties combined. The table shows that the mean percentages of beech infected for the various counties range between 86.5 and 92.7, with a mean for all counties of 89.6%, and with small standard errors throughout. These percentages are shown graphically by the vertically ruled bars in Fig. 3. Further, the percentage of infection for each plot is extremely close to the mean for all plots. A measure of this uniformity is given by the probability odds in the table, which show how often random sampling (as defined in the footnote to the table) would give greater deviations from the mean than those found. The odds for each county and for all counties are greater than 100 to 1, which means that the present deviations are exceedingly small; they are, in fact, even less than would be expected by random sampling alone. The reason for this is not clear; it may conceivably be due to bad sampling methods or to fallibility in detecting the lightest infections.† Since the percentage of infection for each plot is so close to the mean for all plots, there is probably no need for further analysis of the data with a view to correlating differences in the percentage of trees infected with possible environmental or other factors. This does not preclude the possibility that sorting the trees in each plot according to size and other characteristics would show significant differences in the percentage of trees infected.

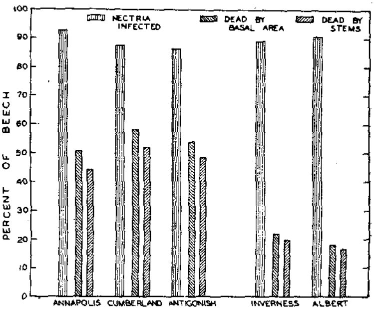

FIG. 3. *Percentages of beech infected by Nectria (according to number of stems), and dead (according to number of stems, and according to basal area), in each of the five counties containing cruise areas.*

It appears from Table III that the areas examined in the five counties do not differ significantly in the percentage of beech infected by *Nectria*, and one might conclude from this that the disease is equally advanced in all. But these figures tell nothing of the severity of the infection on individual trees; that is, they do not distinguish among trees with only scattered or

†*After the completion of the computations, it was realized that χ^2 should have been calculated on the basis of the discrepancies of both infected and uninfected trees from expectation, and, instead of the above formula, the method in Fisher (1930: pp. 82-90), section 21, examples 11 and 13, should have been employed. With χ^2 increased accordingly by a factor of 10, the values of P obtainable from Fisher's table of χ^2 should be decreased materially. The probability odds would be reduced accordingly to more reasonable values, probably indicating that the deviations of individual plot percentages of beech infected by Nectria are not indeed less than would be expected by random sampling alone, but somewhat greater. This opens the possibility that further analysis might show correlations of infection with ecological differences between plots. Owing to the present inaccessibility of the raw data, the recomputations of χ^2 must be postponed.—Author, Kew, March 1934.*

incipient lesions, and trees whose entire trunks are surfaced with infected bark from a continuous system of coalesced lesions studded with perithecia and trees which have already died as a result of infection. In order to bring out differences between counties in the distribution of severity of infection, it was necessary to classify the *Nectria*-infected trees on the basis of severity of infection and to compare the distributions of percentages of infected trees in each class for the different counties. Such a comparison is presented in Table IV.

It will be seen from Table IV that the distributions of the percentages of beech with light, medium, and heavy infection are very similar for the three counties on the mainland of Nova Scotia; not only are the mean percentages themselves close in each class, but the odds expressing the probability of significance of differences in distribution, based on the actual tree counts, are small. The same situation holds for the two grouped counties in Cape Breton and New Brunswick. But the important fact emerges that the distribution for the Nova Scotia mainland differs from the distribution for Cape Breton and New Brunswick, and computation shows the probability of significance of difference to represent odds of more than 100 to 1. This is, of course, as was expected from accounts of the history of the disease in the Maritime Provinces and from preliminary observations. That is, in the examined areas on the Nova Scotia mainland infected for about 10 years, the mean percentage of the infected beech with light infection was found to be 7.4, with medium infection, 18.2, and with heavy infection, 74.4; whereas in the areas examined in Cape Breton and New Brunswick, infected for about four to six years, the mean percentage of the infected beech with light infection was 24.2, with medium infection, 31.4, and with heavy infection, 44.4. In other words, the percentage of infected beech with heavy infection was greater in the region attacked for approximately 10 years than in the region attacked for only four to six years, which means that this difference shows a satisfactory correlation with the period during which the disease is known to have been active in each of the two regions. These differences are shown graphically in Fig. 4.

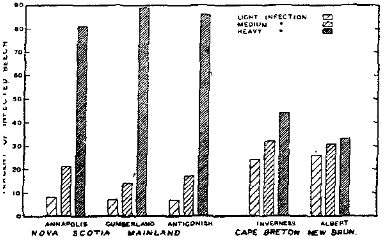

FIG. 4. *Percentages of Nectria-infected beech with light, medium, and heavy infection, in each county containing cruise areas.*

The most striking index to the effects of the disease, however, is actual mortality at time of observation. Mortality can be expressed in two ways, either as (1) the percentage of the total number of beech trees (stems) dead, or as (2) the percentage of the total volume of beech wood dead indicated by the

TABLE IV

SEVERITY OF INFECTION OF *Nectria*-INFECTED BEECH, BY COUNTIES AND REGIONS

County or region	Mean per cent of total beech infected	Number of beech infected by *Nectria*	Per cent of infected beech			Probability of significance of difference in distribution of severity of infection*			Approximate duration of general infection in each region
			Lightly infected	Medium infected	Heavily infected	Between adjacently tabulated counties	Between Annapolis and Antigonish	Between regions	
Annapolis, N.S.	93.3	645	**8.1**	**21.1**	**70.8**	Between 49 and 99 to 1	Between 8 to 2 and 7 to 3	> 100 to 1	10 years
Cumberland, N.S.	88.0	411	**6.8**	**14.4**	**78.8**				
Antigonish, N.S.	89.7	304	**6.9**	**17.1**	**76.0**	Approximately 2 to 8			
Nova Scotia mainland, the three counties	90.8	1,360	7.4	18.2	74.4				
Inverness, (C.B.) N.S.	90.3	2,029	**23.7**	**31.6**	**44.7**	Between 1 to 1 and 7 to 3			4-6 years
Albert, N.B.	90.4	668	**25.7**	**30.6**	**43.7**				
Cape Breton and New Brunswick	90.3	2,697	24.2	31.4	44.4				

**These values are obtained by computing χ^2 according to a method in Fisher (1930 : p. 84-85, ex. 11) and converting into odds the values of P obtained by entering the χ^2 table.*

ratio which the basal area* of the dead beech bears to the basal area of all the beech. The first method gives a satisfactory index to the effect of the disease on the number of trees in a stand but, because size of tree is ignored, does not give an indication of the actual amount of damage in volume of timber. The mortality figures have been computed by both methods, taking the mean of the percentages for the individual plots, and are presented in Table V. The table shows that the data fall naturally into two groups which differ sufficiently from each other in the mean percentage of trees killed to justify their separation, but each of which is sufficiently homogeneous internally to warrant its handling as a group. Therefore, in subsequent analysis of mortality (Section VIII) the groups are treated separately. One group includes areas in Annapolis, Cumberland, and Antigonish Counties, Nova Scotia, with a mean percentage of beech dead of 48.0 by stems and 54.1 by basal area; the other, parts of Inverness County in Cape Breton Island, Nova Scotia, and Albert County, New Brunswick, with a mean percentage of beech dead of 19.2 by stems and 21.1 by basal area. The difference in percentage of mortality seems to be correlated with differences in the duration of the period in which the trees have been attacked by the pathogens, a situation reflected by the data on distribution of severity of infection (Table IV, Fig. 4).

It is further indicated by the table that for the regions examined the losses have been highest in Cumberland County, less in Antigonish County, and still less in Annapolis County. This suggests that the disease has been active longest in Cumberland County, for a shorter period in Antigonish County, and for a still shorter one in Annapolis County. This is in direct agreement with the data on distribution of severity of infection (Table IV, Fig. 4) and with the observations of foresters and others, and is in keeping with the fact that the disease has spread in all possible directions from the vicinity of Halifax. Similarly, the figures in the table are in agreement with the data on severity of infection and with observations in Cape Breton and New Brunswick, both indicating the presence of the disease for a somewhat longer period in parts of Cape Breton than in Albert County, New Brunswick.

Standard errors of the mean percentages of mortality for each county have been computed, and are low. The odds (see footnote 2 to Table V), contrary to those for percentage of beech infected, show the probability, that additional samplings would deviate more from the mean than those found, to be extremely slight, and thus indicate that the differences in percentage of beech killed from plot to plot are significant. This is expected from the fact that individual plot percentages range from 0 to 100, and suggests that the data for mortality should be analyzed further with a view to correlating the present percentage of beech killed with possible environmental or other factors. This subject is discussed in Section VIII.

***Basal area is total area at breast height (4.5 ft.) of the cross sections of all the stems. It is computed from the diameter by the usual formula: $\Sigma A = \Sigma\left(\frac{\pi d^2}{2}\right)$, where A is the area, π is 3.14, and d is diameter.**

TABLE V

PERCENTAGE OF BEECH DEAD, BY STEMS AND BY BASAL AREA (PLOT-PERCENTAGE MEANS), BY COUNTIES AND REGIONS

Regions		Nova Scotia mainland				Cape Breton and New Brunswick		
Counties		Annapolis	Cumberland	Antigonish	The three counties	Inverness	Albert	Both counties
Mean plot per cent beech dead by stems	Per cent beech dead	**44.2** ±3.7	**52.0** ±4.2	**48.6** ±3.9	**48.0** ±3.5	**19.9** ±2.0	**16.7** ±2.0	**19.2** ±1.7
	Probability odds*	<1 to 99	>1 to 99	>1 to 99	<1 to 10,000,000	<1 to 10,000,000	1 to 1	<1 to 10,000,000
Mean plot per cent beach dead by basal area	Per cent beech dead	**50.6**	**58.2**	**53.9**	**54.1**	**22.0**	**18.0**	**21.1**

*These values were obtained by computing χ^2 from the actual numbers of trees killed in each plot and the numbers expected, with a mean percentage of trees killed of 50 for the areas in mainland of Nova Scotia, and of 20 in Cape Breton and New Brunswick. The forumla is: $\chi^2 = \Sigma\left(\frac{x^2}{m}\right)$, where x is the difference between the actual and expected numbers of trees killed, and m is the expected number. Where n (the number of sample plots, minus 1) was 30 or less, the value of P was read from the χ^2 table in Fisher (1930) and these probabilities were then converted into odds. Where n was more than 0, the formula $\sqrt{2\chi^2} - \sqrt{2n-1}$ was used; and the value of P was read from Fisher's table of x.
The values in this column measure the probability that additional samplings from the same population would show deviations as great as, or greater than, hose in the present sampling.

The data on mortality are presented graphically by the diagonally ruled bars in Fig. 3, which shows additionally that although the time factor has little effect on the percentage of beech infected by *Nectria*, it bears a definite relation to mortality. It is evident that under conditions in the Maritime Provinces inoculation and infection take place rapidly but that mortality, although beginning promptly, extends over a period of years; it attained, in the areas examined, a magnitude of approximately 20% in four years and 50% in ten years. This is based on the reasonable assumption that climatic or other differences between the regions are not significant.

Table V shows further that mortality by basal area varies closely with mortality by stems, but is slightly greater (owing to omission of stems under four inches d.b.h.). If it could be shown that this correlation is constant and high for the data in the present survey, it would not be necessary to compute both figures in subsequent analyses of the data, since the values based on stems would be a sufficiently good measure of the damage expressed as basal area. Accordingly the values for each of the two regions were entered on a scatter diagram (Figs. 5, 6), on which the ideal perfect-correlation line is

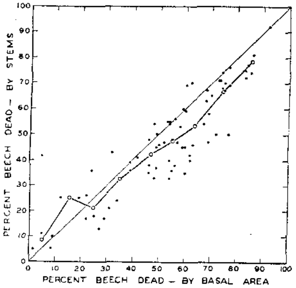

FIG. 5. *Comparison of percentage of beech dead according to number of stems, with percentage dead according to basal area; for plots in Nova Scotia mainland.*

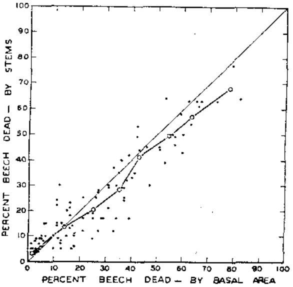

FIG. 6. *Comparison of percentage of beech dead according to number of stems, with percentage dead according to basal area; for plots in New Brunswick and Cape Breton.*

represented by the diagonal. The values according to basal area were arranged in 10% classes and class means obtained for basal area and for the stem values of the plots in each basal-area class. These mean points are plotted as circles on the diagram and show graphically the degree with which the mean of the actual plot points approximates to the ideal. The mean percentages of mortality by both methods, according to 10% basal area classes, are shown in Table VI.

It will be seen from Figs. 5 and 6, and Table VI that mortality (percentage of beech dead), expressed as stems, is closely correlated with mortality expressed as basal area. A statistical evaluation of this correlation is afforded by Pearson's coefficient of correlation r, which is computed from the data for

TABLE VI

PERCENTAGE BEECH DEAD BY STEMS: COMPARED WITH PERCENTAGE DEAD BY BASAL AREA, EXPRESSED AS CLASS MEANS ON THE BASIS OF PLOT PERCENTAGES

Region	Percentage beech dead by	Per cent classes of beech dead by basal area										Correlation coefficient r *	Probability of significance of r †
		0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100		
Nova Scotia mainland	Basal area	5.2	15.5	24.8	35.0	47.0	55.1	63.8	74.2	84.2	92.0	0.892 ±0.0025	Much > 100 to 1
											85.5		
	Stems	8.7	25.0	21.2	32.6	42.3	47.5	53.5	66.5	75.8	92.0		
											78.5		
Cape Breton and New Brunswick	Basal area	1.7	14.0	25.0	35.1	43.0	54.6	63.3	76.0	82.0	—	0.922 ±0.0149	Much > 100 to 1
										78.0	—		
	Stems	3.1	13.5	20.3	28.1	41.3	49.4	56.9	70.0	64.0	—		
										68.0	—		

*r *is Pearson's coefficient of correlation and has been calculated by means of the correlation data sheet of Thurstone (1925: p. 202) according to the formula:* $r = \dfrac{\dfrac{\Sigma xy}{N} - (dx \cdot dy)}{\sigma_x \cdot \sigma_y}$. *The values of r range between* −1.0 *and* +1.0, −1.0 *indicating a perfect negative correlation,* 1.0 *a perfect positive correlation, and* 0.0 *no correlation whatever. The figure following* ± *is the standard error of r, calculated according to the formula in Fisher (1930: p. 158):* $\sigma_r = \dfrac{(1-r^2)}{\sqrt{n-1}}$.

†*These odds measure the probability that the computed value of r could not have arisen by chance, and thus are a measure of the degree of significance attaching to r. They are secured by computing t according to the formula in Fisher (1930: p. 159):* $t = \dfrac{r}{\sqrt{1-r^2}} \cdot \sqrt{n'-2}$ *and entering Fisher's table of t with n equal to two less than n', the number of the paired observations.*

the individual plots; the values of r for each region are convincingly high and their standard errors assuringly minute. Another method of expressing the significance attaching to r is the odds that the computed value of r could not have arisen by chance; these odds are seen to be much greater than 100 to 1, and leave no doubt as to the degree of correlation.

In short, these data show that in the samples examined from widely separated regions the numbers of beech infected by the fungal pathogen were uniformly close to 90%, that the severity of infection was greater in regions attacked longer, and that mortality, although ranging for individual samples from 0 to 100%, attained a mean of approximately 20% in recently attacked areas and 50% in places exposed for a longer period. The data thus bear out the assertion that this disease has attained the proportions of a fatal epiphytotic which has already seriously depleted the beech stands of the Maritime Provinces, and represents an imminent danger of great importance to the beech stands of adjacent areas. When it is further realized (1) that mortality increases with size of tree (as discussed in Section VIII) and that, accordingly, the beech in the stand has been almost wholly destroyed so far as its merchantable value is concerned, (2) that loss of the older seed-bearing trees must lead to the curtailment of future reproduction, and (3) that the weakened, dead, and fallen trees provide ideal conditions for the building up of a dangerous host of insects and fungi capable of developing in the slash and weakened trees, it becomes evident that the disease is a serious matter.

VI. Symptomatology

A. GENERAL ACCOUNT

This disease begins with the infestation of the bark by the minute scale insect, *Cryptococcus fagi* (Baer.). The insect feeds by inserting its stylets (proboscis) into the bark, penetrating the corky layer, cork cambium, and phelloderm, and probing the underlying tissues of cortex and phloem, into which saliva is injected, and from which protoplasmic fluids are withdrawn. When populous colonies of the insect feed in localized areas on the bark surface, whole clusters of parenchyma cells are killed. These clusters dry and shrink, causing minute fissuring of the phellem and subjacent tissues. In regions where an abundance of inoculum of the *Nectria* has accumulated, these fissures quickly become inoculation courts and provide satisfactory substrata for incubation and initial infection of tissues normally resistant, by reason of phellar protection, to this fungus. Once established, the mycelium is able to spread beneath the phellem, invading phellogen, phelloderm, cortex, phloem, cambium, and the rays; growth is both inter- and intracellular in the susceptible tissues, but in the cork, in the nests of sclerenchyma of the older phloem, and in the non-living elements of the wood, mycelium is not found.

Light infestations of the insect and isolated fungal infections which do not enlarge rapidly frequently stimulate the suscept to the activation of a trau-

matic secondary meristem. This meristem leads to the formation of a hyperplastic tissue raised above the surface of the healthy bark. If this tissue is successful in cutting off the advance of necrosis with a resistant suberized callus layer, the infected area may be thrown off by the cracking of the margin of the lesion; if unsuccessful, the mycelium continues to radiate from the original infection court, killing all invaded cells and resulting in a disk-shaped circular lesion, somewhat sunken by the shrinkage of the dead tissues and frequently delimited at the margin by the cracking of the cork layer. When the lesion attains a periclinal diameter of one or two centimetres, the mycelium aggregates in the region of the phellogen and forms a band of pseudo-parenchymatous stroma which ruptures the phellem and results in the formation of pustules containing erumpent sporodochial proliferations of the stroma. On these sporodochia the hyaline elongate macroconidia (form genus *Cylindrocarpon* Wr.) are formed in dense clusters which appear to the unaided eye as loose white masses, resembling somewhat on superficial observation the woolly secretion of colonies of *Cryptococcus fagi*. Later, perithecia develop on the same stromata among the bases of the old conidiophores and, with the disappearance of the conidia, protrude from the pustules as globular red bodies.

Meanwhile, the continued advance of the mycelium leads to an extended zone of necrotic tissue which in time includes the cambium and inhibits further meristematic activity. The production of spores and consequent multiplication of the number of infections, combined with their periclinal enlargement, lead to the rapid coalescence of the individual necrotic lesions and the dying of large areas of bark, cambium, and young sapwood. These areas of bark become cracked with drying and shrinking, frequently loosen from the wood, and scale off in great sheets. On younger trees and in exposed locations where the fungus is able to develop less rapidly, where inoculations are less abundant, infections less successful, and fruiting less prolific, the general progress of infection is greatly retarded, individual lesions enlarge less rapidly and rarely coalesce, and a much smaller proportion of the vital tissues of the tree is killed. Under such conditions, continued meristematic activity of the cambium in the healthy areas results in the formation of depressions which are occupied by the lesions, and occasionally of cankers, some of which become eventually buried under callus rolls of new wood and bark.

The continued encroachment of the fungus on the tissues of the conducting and storage system of trunk and larger branches interferes increasingly with their functions and soon produces symptoms of moisture deficiency in the crown of the tree. As the growing season advances, foliage first curls, then dries, and becomes prematurely brown. Twigs and small branches become dry and many fail to produce buds. In the following spring, foliage is absent on many branches and the bark begins to crack. On other branches small chlorotic leaves develop, but they shrivel with the advent of hot dry weather. At this stage, large portions of the crown are already dead, the bark is loosening in patches on the trunk, and the end is near. The openings produced in

the crown canopy now retard the activities of the fungus by reducing the moisture both in the lower atmosphere and in the bark; but they also remove moisture more rapidly from the soil and leave less available to the tree. A few of the heavily infected individuals are able to survive this drought and profit by its inhibitory effect on the fungus. Most trees soon die.

B. SIGNS

The Insect

In an infested area, countless numbers of minute yellow larvae of *Cryptococcus fagi* can be detected by scrutiny of the bark during the months of August and September. By late autumn many of these larvae settle in sheltered positions on the bark, insert their slender and powerful stylets, and secrete from numerous glands a wax which is exuded in long curly threads and soon envelops them in a fluffy white down. The numbers of insects increase year by year and individual insects are replaced by colonies; the colonies grow numerous and populous, and the secretions of white wax form a continuous mass of "wool," interspersed with the shrivelled bodies of annual generations of exhausted females. Eventually under favorable conditions the trunk and branches appear at a distance as though coated with driven snow. Although in sheltered stands the insect can feed on any portion of the trunk and on the undersides of the larger branches, the densest masses of "wool" occur in lenticular crevices, in cracks and wounds, under mosses and foliose lichens, and in the abaxillary angles of the lower branches. A light young infestation in lenticular crevices and old superficial wounds on the trunk of an ornamental specimen of *Fagus sylvatica* is shown in Plate II, Fig. A; an older and heavier infestation on a forest beech *(F. grandifolia)*, still following in general the lenticular crevices, but covering almost all except heavily corked areas, appears in Plate II, Fig. B; somewhat enlarged views showing the depths which the woolly mass attains with increasing infestation may be seen in Figs. C and D. Still greater enlargements show how on exposed bark the colonies begin in lenticular crevices (Plate II, Fig. E), and how the individual threads curl and interlock to form a deep but light and resilient canopy over the insects nestling beneath (Plate II, Fig. F). With advent of the fungus, the tissues on which the insect depends for nourishment are killed, the current generation of insects dies, and in time most signs of the infestation disappear.

The Fungus

Following infection of the bark in a minute fissure developing after feeding by the insect, the mycelium radiates periclinally under the phellem. After a time pseudoparenchymatous aggregations of hyphae are formed in the region of the phellogen and can be seen as orange-colored masses by shaving the corky layer. Later, they become erumpent in a ring, from one to two centimetres in diameter, of white sporiferous pustules (Plate III, Fig. A), this ring being situated centrad of the periphery of the mycelium. The corky layer of the bark is broken usually without the loss of the upturned edges,

which remain surrounding the sporodochium (Plate III, Fig. B). The maturing macroconidia are released from the conidiophores and borne away by wind and rain; meanwhile, perithecial initials have been forming at the surface of the stroma and with the passing of the conidia are elevated among the remnants of conidiophores, and stand in clusters that obscure the stromata from which they spring (Plate III, Figs. C, D). With heavy inoculation and conditions propitious to uniformly rapid inoculation, incubation, infection, and fruiting, the numbers of perithecia developed are so enormous that the trunk of a severely attacked beech, especially when wet, sometimes appears red with perithecia. This is particularly striking when fresh perithecia form promptly on the areas between the original circular lesions, newly infected by the advancing mycelia, so that square metres of bark surface are uniformly studded with close packed clusters of these small, bright red fruiting bodies.

As asci and ascospores mature, the perithecia swell, rising above the bark and crowding one another, so that many central ones either remain lower than the majority or else become laterally compressed, and many marginal ones are forced beyond the original limits of the pustule (Plate III, Figs. D, E). The apex of the perithecium becomes slightly drawn out into a papilla which contains a structurally organized ostiole through which the ascospores are discharged. When the perithecium first becomes wet, discharge is violent, but with the passing of time and the thorough wetting of the bark and perithecium the spores are forced by swelling of the ascus into the ostiolar canal in such numbers that they jam and ooze out, first in a spore plug (Plate IV) which becomes a delicate curling tendril, later as a formless mass which covers the apex of the perithecium and frequently remains with drying *in situ* until the next rain. When most of the ascospores have been discharged the upper half of the perithecium collapses and sinks into the lower half, much like a deflated rubber ball, so that the empty perithecium sometimes resembles superficially the fructification of a discomycetous fungus (Plate III, Fig. F). The color of the first emergent perithecium is a bright red, of the old discharged one, a dark fuscous red. Within a year after discharge, weathering usually reduces the cluster of perithecia to a few drab, dark colored, formless remnants.

Trees weakened by prolonged insect infestation and by *Nectria* infection are frequently attacked by weak parasites and saprophytes which may increase the pathological effects and produce disintegration of the infected tissues. The most common of such organisms in the Maritime outbreaks are *Scolytid* bark beetles, and fungi. A beetle is occasionally seen crawling on the surface of the bark and boring into the centre of a young circular lesion; more often only the entrance to its tunnel is visible, usually plugged with a core of frass which may protrude for a distance of almost a centimetre from the surface of the bark. The commonest of the fungi are species of *Hypoxylon* which are occasionally able to grow in living bark and produce broad shallow cankers; their brick red to brown or black superficial stromata are conspicuous over large surfaces on dead areas of bark. Other fungi whose fruiting

bodies are encountered commonly on dying or dead bark are members of the Thelephoraceae (especially species of *Stereum*), Dacryomycetales, Agaricaceae, and Polyporaceae (especially *Fomes fomentarius* L. ex. Gill., *F. igniarius* L. ex. Gill., and *F. applanatus* Pers. ex Wallr.).

C. MACROSCOPIC SYMPTOMS

ON INFECTED BARK

Dying of lichens and mosses. Discoloration and death of the mosses and foliose lichens which abound on the trunks of beech in the Maritime forests are among the first symptoms of infection visible to the unaided eye. The first colonies of the insect and the first fungal lesions develop on the bark beneath moss and lichen thalli. The epiphytic habit of these plants precludes the possibility that mere death of their substrata exerts a lethal influence; a more likely, but as yet unsubstantiated, explanation is that the fungus itself produces diffusible substances toxic to the epiphytes; the production of such toxins is suggested also by the death of bark cells adjacent to fungal hyphae but which the hyphae do not penetrate, and by the death of cells some distance in advance of the mycelium. When infection takes place under conditions not highly favorable to the fungus, a lesion frequently remains restricted to the area covered by the lichen thallus, becoming and remaining depressed with the continued growth of the surrounding healthy tissues (Plate V, Fig. D).

Bark lesions. Death of the infected bark is followed by desiccation and shrinkage, so that when seasonal changes cause a temporary cessation of mycelial advance the circular zone of necrotic tissue becomes slightly depressed and sometimes cracked both internally and about its margin; simultaneously, the pressure of stromatic aggregations produces pustules whose edges are formed by upturned phellem, first in a ring some few millimetres within the margin of necrosis, and later throughout the central zone (Plate V, Fig. B). When the mycelium resumes growth the lesion enlarges periclinally and coalesces with adjacent infections, resulting in large, irregularly shaped, necrotic areas which become covered indiscriminately with pustules and develop larger and deeper cracks, penetrating ultimately to the cambium; on such areas the limits of the original circular lesions are sometimes still discernible (Plate V, Fig. A). Finally, the drying and shrinking of extensive areas of bark produces long vertical cracks connected here and there by horizontal ones; loosening of the bark from the wood by death of the cambium leads to the scaling off of roughly rectangular sheets which fall away, exposing the drying and splitting wood of the trunk (Plate VI, Fig. E).

The local necrosis and fissuring produced by the insect expose the susceptible inner tissues to infection not only by the pathogenic *Nectria* responsible for most of the injury but to a host of other organisms. Certain of these are purely saprophytic and grow only in the dead tissues, disintegrating, digesting, and fermenting them to a watery or slimy mass which oozes from the surface of the bark and seeps down as a discoloring liquid. Such a lesion

(slime flux) has a characteristic odor reminiscent of certain organic acids. The decomposition products of these activities evidently contain substances toxic to the living tissues of the bark, because discoloration and death of the cells extend for a considerable distance beyond the area occupied by the micro-organisms and may ultimately involve several square centimetres of bark surface. A cavity penetrating to the wood is common at the centre of the slime flux and is a favorite point of entry for *Scolytid* beetles and rendezvous for sow bugs (Oniscoida).

Cankers. On younger trees and on bark in open stands the number of infections is much smaller than in the dense forest. The same factors that determine this situation apparently also weight the odds in the combat between pathogen and suscept in favor of the latter. Individual lesions remain more nearly circular, enlarge slowly or not at all. Occasionally they are thrown off by formation of a corky layer which circumscribes and isolates the infected tissues, raising them as a disk which may rupture (Plate V, Fig. C), and eventually causing them to slough off. More often the continued growth of surrounding healthy meristems encircles the infected zone with a callus of new bark and wood. In the early stages of the process, pustules and fructifications of the *Nectria* can still be seen on the infected bark. Later, these signs and symptoms disappear and the lesion becomes a deeply depressed cavity surrounded and partially or completely healed over with callus. Stems supporting many such lesions become pitted and gnarled (Plate V, Figs. E, F). This process does not usually result in a typical scalariform canker, probably because the fungus is unable under such conditions to keep pace with the active meristems of the suscept.

Internal necrosis. The infected tissues of the bark beneath the phellem change in color from their normal pale yellow to a pale orange red and later to a dark red which becomes tinged with brown on drying. The first infections are usually superficial so that shaving the bark to a depth of three or four millimetres removes all necrotic tissue. In time, with the spread and penetration of the mycelium, the necrotic areas coalesce and penetrate in the old centres into phloem, cambium, and sapwood. Removing all tissues above the cambium in such an area exposes the surface of the sapwood (Plate VI, Fig. D) which has been reached in a number of points by the advancing mycelium, but in only one of which a sufficiently large area of cambium has been destroyed to leave a disk of red necrotic tissue surrounded by newly formed sapwood. With active infections, areas such as this one enlarge and coalesce with others until the conducting tissues are effectively interrupted by a series of vertically overlapping cambial lesions. This condition interferes with conduction of water and inorganic salts, of elaborated organic foods, and with storage of the latter in both phloem and xylem, all exerting a critical inhibitory influence on the normal metabolic and meristematic activities of the entire tree.

On young or isolated trees the individual circular lesions enlarge but little and are frequently walled off by callus and then either sloughed off by being

raised, or else buried. A series of views of two small sunken lesions on a young stem is shown in Plate VI, Figs. A, B, C. Fig. A shows the surface aspect with the bark cracked across the face and with the borders raised in rolls of newly formed bark and wood. Shaving to the depth of the bark in the depressed necrotic areas (Fig. B) exposes the new wood; the necrotic areas are dry and brown-black; the borders which still support active infection are light red; and the surrounding healthy wood is a pale yellow. At a depth of three eighths of an inch (Fig. C) the lesions are still visible in the wood and show by their dark color and by the curving contour of the healthy wood outside that they have been present but relatively inactive for a number of years. A similar condition exists in lesions on older trees which have become exposed subsequent to infection by the opening of the crown canopy.

On the Crown

The destruction of protective, vascular, and storage tissue-systems of the trunk leads to a progressive killing of the tree, accompanied by striking symptoms in its crown. When they first appear late in the growing season, these symptoms are usually diagnostic of water deficiency. Leaves, which could be kept turgid earlier in the season, curl and become prematurely brown. The twigs dry; no buds are formed; the dead leaves adhere and weather through the winter. In the following spring, these and other branches remain bare and twigs begin to break off. Other branches, undersupplied with stored food, put out chlorotic undersized leaves which may or may not survive the summer. The large crown openings which result expose the remaining foliage to sun and wind to a greater extent than normally; this increases even more the demands on a rapidly disintegrating vascular system. The partial girdling of beech by porcupines, common in parts of the Maritime Provinces, serves to intensify the crisis. With the loss of foliage and stored organic food materials, the roots dry and decay, conduction comes to an end, the remaining uninfected living tissues die of water deficiency, and the progressive killing of the tree is complete. A *Cryptococcus*-infested beech ridge in Inverness County, Cape Breton Island, which was infected heavily by the *Nectria* but a year or two before being photographed, is shown in Plate I. Certain of the trees died during the previous year and are leafless. Others were able to leaf-out, but renewed activity of the fungus completely disrupted vascular activity and the foliage dried and became brown. Others still support a chlorotic verdure, and only a few show vigorous healthy crowns. Other views in attacked stands appear in Plate VI, Figs. F, G. Fig. G, taken along a newly cut highway in Cumberland County, Nova Scotia, shows young and old beech plucked by the disease, with nothing but maples remaining. Fig. F is from Annapolis County, Nova Scotia, and shows a similar condition.

D. MICROSCOPIC SYMPTOMS

Feeding by Cryptococcus fagi

Although there has been considerable study of the histological aspects of the effects of sucking insects on leaves and stems, only Hartig (1880) and Boodle

and Dallimore (1911) have reported observations on *Cryptococcus fagi.* Hartig apparently made no study of the relation of the suctorial apparatus of the insect to the plant cells, but noted that the stylets are able to penetrate to the green bark parenchyma and stimulate the production of a radially arranged, thin-walled gall parenchyma by meristematic activity of the parenchymatous elements of bark and phloem. In young trees this leads to a tearing of sclerenchyma nests and the formation of superficial galls. Small galls remain closed; larger ones burst and expose the wood, which dies and, by the accumulation of decomposition products, develops longitudinal brown streaks. In older trees the greater sclerenchymatization prevents gall formation, but the killing of parenchyma cells produces browning and drying, which lead to tearing of the bark. Boodle and Dallimore (1911) observed the sucking tubes of the insect in the outer tissues of the bark, sometimes reaching to a depth of one millimetre. They noted differences in the amount of sclerotic tissue and of cell contents in the phloem, but failed to correlate the presence or abundance of *Cryptococcus* with these conditions.

Although the writer encountered technical difficulties in preserving the insects *in situ* during washing and embedding, in softening the sclerenchymatous tissues without injury to the delicate parenchyma, and in sectioning, a satisfactory technique was ultimately devised which yielded thin sections of well preserved material. Blocks of infested bark were cut from the tree and either the top surface dipped into hot wax or celloidin or the entire block wrapped in perforated waxed paper. Fixation was best in weak chromo-acetic solution and in Carnoy's fluid. After removal of the reagent the siliceous materials were partly dissolved in hydrofluoric acid diluted with 30 or 50% ethyl alcohol. After washing, and dehydration by the butyl alcohol series, the blocks were embedded in nitrocellulose (preferably Mallinckrodt's Pyroxylin) and sectioned with a sliding microtome. The most useful stains were safranin and light green followed by mounting in balsam, and acid fuchsin in lactophenol.

The stylets, or portions of the suctorial apparatus which are inserted into the plant, consist, as with other Homoptera, of the maxillae and mandibles modified into four slender and flexible grooved filaments which are held appressed to form a double-tubed pump. One channel pumps saliva from the salivary glands; the other sucks up fluid materials from the punctured plant cells, mixed with saliva. The stylets attain a length in adults of almost 1500μ. They can reach into the outer phloem in young bark, but extend most commonly into phelloderm, adjacent cortex, and pericycle. The phellar layer is usually penetrated perpendicularly without visible tearing and apparently without being dissolved (Fig. 7). In the phelloderm and within, the course is not fixed, being sometimes parallel to the axis of the stem, and sometimes tortuous. An adult rarely penetrates the corky layer more than once, but probes internally in all directions by branching from the original path and from laterals. The walls of living cells, unless sclerotic, do not obstruct the stylets; penetration is intra- as frequently as intercellular

(Figs. 7, 8; Plate VII, Figs. D, F, G). Only when a nest of stone cells is encountered do the stylets either stop or attempt to circumvent the obstruction (Plate VII, Fig. C); occasionally progress is made between the cells of such a nest (Plate VII, Figs. A, E).

The course of an insect's probing is fortunately marked by the formation of a permanent structure, the stylet track. This consists of the hollow or precipitate-filled stylet-sheath, which remains after the stylets are withdrawn, as a permanent record of their course. The composition of this material is not known; it stains strongly with both safranin and acid fuchsin. The sheath appears to be two-layered, consisting of a narrow, uniform inner lining which is in contact with the stylets while they remain, and a thick irregular outer material, deeper over the portions of the sheath within the plant cells than in the intercellular portions, and deeper in living parenchyma cells than in dying cork cells (Figs. 7, 8). The inner lining sometimes protrudes somewhat above the surface of the bark (Fig. 7). These characteristics suggest that a component of the lining is secreted by the insect and covers the surface of the stylets, while the outer material is precipitated by the reaction of the contents of the punctured cells with this material or with a constituent of the saliva.

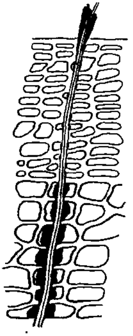

FIG. 7. *Stylets and stylet track of Cryptococcus fagi in periderm of Fagus grandifolia.*

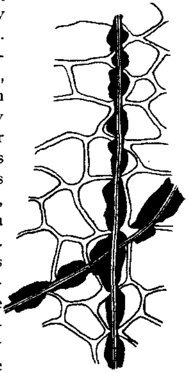

FIG. 8. *Stylets and stylet tracks in bark parenchyma.*

The contents of punctured cells are shrunken and killed, but no pathological effects seem to extend at first beyond the injured cells, suggesting that the saliva contains no violently toxic constituents capable of diffusing from the immediately probed point into neighboring cells. With continued feeding of large numbers of individuals at a localized point, the entire area dies and shrinks and cracks. The area becomes brown and the browning extends to surrounding unpunctured cells. The whole is frequently surrounded by a meristem which builds a wall of radially arranged parenchyma cells; these sometimes become suberized, isolate the area, and cause the ultimate death by starvation of the feeding insects. In spite of the protection afforded the insects by lenticular shelves of cork (Plate VII, Fig. B), the lenticels on sheltered bark are not probed as abundantly as the interlenticular surfaces, probably because the greater depth of loose phellar sheets in the lenticels increases the difficulty of reaching succulent tissues.

INFECTION BY NECTRIA

When ascospores or conidia of the *Nectria* are deposited in the cracks which form in the bark subsequent to feeding by *Cryptococcus fagi*, their germ hyphae are able to enter the exposed parenchyma tissues of phelloderm and cortex and grow both inter- and intracellularly, occupying the fissures which have resulted, and sending hyphae into the living tissues. The nests of stone cells are occupied only intercellularly but other cells are entered and killed (Fig. 9). The walls of the dead cells collapse and form a moist red-brown mass, the interstices of which become filled with broad, irregularly swollen, short celled hyphae of a pale yellow color. Tissues some distance beyond the visible limits of the mycelium die and become brown, suggesting toxic substances, produced by the action of the fungus on the bark cells. Meanwhile, mycelial aggregations form dense pseudoparenchymatous stromata in the phellogen and press outwards against the phellem which eventually ruptures (Plate VIII, Figs. A, B, D). As the stroma rises to form a sporodochium, indications of its hyphal nature are obliterated and the cells become rhomboidal in longitudinal outline and form a pseudoparenchyma. The topmost cells elongate and become conidiophores on which the long, slightly curved, four–seven-septate, hyaline macroconidia are borne (Plate VIII, Figs. C, E). As the conidia are released, perithecial initials take form in the upper portion of the stroma and become elevated by an elongation of their basal cells (Plate VIII, Fig. F).

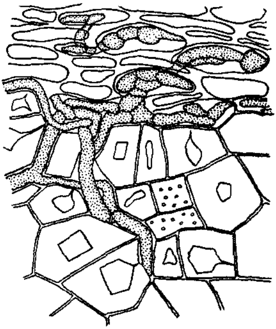

FIG. 9. *Nectria mycelium penetrating bark-parenchyma cells, but intercellular among stone cells.*

VII. Etiology

A. THE INSECT

NOMENCLATURE

Common Names

By far the greatest number of references to *Cryptococcus fagi* have been made in Germany and Great Britain. In Germany, probably the earliest common name known to be applied to the insect is in the reference which Ratzeburg (1869) made to a species of "Schildlaus" (genus *Coccus*) on beech, discovered by R. Hartig. Hartig himself referred to it as the "Buchenwolllaus" (1878) and the "Buchen-wollaus" (1880); the latter name was used by Altum (1881); similar designations were "Buchen-Woll-Laus" (Schröder 1897), "Buchen-Wollaus" (Bertelsmann 1913 a), and "Buchenwollaus" (Bertelsmann

1913 b). Sinz (1913, 1914) used the latter two names erroneously for the aphid, *Lachnus exsiccator* Alt. (*L. fagi* L.), and drew attention to the confusion which can arise in common German names of the bark infesting coccid *Cryptococcus* with the leaf sucking aphid, *Lachnus*. The name "Buchen-Wollschildlaus" was first used by Judeich and Nitsche (1895) and later by Nüsslin (1905, 1913) and by Eckstein (1920); Rhumbler's (1914 a, 1914 b, 1915, 1922, 1931) "Buchenrinden-Wollaus" is perhaps the most descriptive and least confusing of the German names.

In Holland the insect has been called "Beukenwolluis" (Anon. 1921), and in Sweden, "Boksköldlusen" (Trägårdh 1921).

In Great Britain the insect has been known longer than in Germany, although the first published reference at hand, by M'Intosh (1849), spoke of it as a white, rapid-spreading fungus. As late as 1860 Berkeley still mistook it for a fungus, although he later corrected this impression (Berkeley 1865) and two years later referred to the insect as the "coccus" (Berkeley 1867). It had been recognized as an insect as early as 1850 by some (C.C., M'Intosh, Hardy) and was rather well described by Hardy (1850). Nevertheless, it was not until 1898 that Miss Ormerod pointed out that no common English name was yet in general use, and suggested "beech-bark 'felt-scale'," a name subsequently used, to the present writer's knowledge, only by Gillanders (1908). This name apparently met with little approval, for two years later Newstead (1900) called it the "felted beech coccus"; and this name has since been in common use (Boulger 1907, Webster 1908, Munro 1925, Forestry Commission 1926). Other British names are "woolly scale of the beech" (Carpenter 1903), "beech-felted scale" (MacDougall 1908), and "beech coccus" (Boodle and Dallimore 1911).

In Canada the first reference is by Braucher (1914), who quoted from a letter in which Dr. L. O. Howard stated that a Mr. Sanford had identified the specimen in question as the "European felt scale." The Dominion Entomologist used Newstead's name "felted beech coccus" (Hewitt 1914), as did Gorham (1929) and Gorham, Walker, and Simpson (1929). Subsequent designations were "the imported beech bark-louse of Europe" (Motherwell 1926), "beech bark louse" (Graham 1927), "European beech coccus" (Swaine 1930), "beech coccus" (Balch 1932, Ehrlich 1932 b).

In the United States the common name approved by the American Association of Economic Entomologists (1930) is "beech scale." This name has accordingly been used by Schaffner (1931), Collins (1931, 1932), Peirson (1932 a, b), and Ehrlich (1932 a, 1933), although the woolly character of the waxy secretion, which is not very common in scale insects, seems, in the opinion of the present writer, to justify and call for the name "woolly beech scale."

Latin names

It has been generally conceded by entomologists that Baerensprung (1849) was the first to recognize the woolly beech scale as an insect and named it

Coccus fagi. But entomologists seem to have overlooked the fact that the mycologist Fries (1832) had observed the woolly secretion of this insect some 20 years previously, had assumed that it was fungal in nature, and had published a name and description. He placed it in his genus *Psilonia*, Fungi Imperfecti, class Hyphomycetes, order Sepedoniei, and named it *Psilonia nivea*. This regrettable error was recognized early by some mycologists, but was propagated unknowingly by others through the remainder of the nineteenth century. Although the writer has not personally examined type material and although Fries mentioned spores, the writer is convinced that Fries actually had before him none other than the "wool" of *Cryptococcus fagi*, occupying crevices in the bark of *Fagus sylvatica*.

In 1838 Libert distributed, in her "Plantae cryptogamicae quas in Arduenna collegit," specimens marked *Psilonia nivea* Fries; the writer has been privileged to examine these in the Farlow Herbarium, and found no fungus but the "wool" and shrivelled bodies of the beech scale. A second notice of *Psilonia nivea* appeared in Fries (1849). Berkeley and Broome include *Psilonia nivea* Fries among British fungi and note that it is "remarkable for its curled flocci, which sometimes resemble unrolled spiral vessels" (1859 : p. 361). It is mentioned again the following year (Berkeley 1860). Following this, Berkeley evidently became aware of the entomological nature of his material: in 1865 we find him writing to a correspondent of the Gardeners' Chronicle: "The white substance on your Beech bark has been included amongst Fungi under the same of Psilonia nivea, but it is an insect production . . . . " (Berkeley 1865 : p. 776). Two years later he quoted a letter from Professor C. C. Babington in which it is stated that he found the bark of his beeches covered with the "coccus," "which produces a quantity of white smooth hyaline threads which are often mistaken for a Fungus, and were described as such under the name of Psilonia nivea by Professor Fries. . . . . " (Berkeley 1867 : p. 235-6). In the same year Kickx published a description, under his family Byssoïdées, of the "fungus" *Psilonia nivea* Fries, obviously based on the original description and the Libert exsiccatus, and appended the following remark: "On trouve souvent entremêlées aux acrospores . . . . des globules microscopiques, qui paraissent être des conidies" (Kickx 1867 : p. 285). In 1871 Cooke stated: "*Psilonia nivea* Fr. is clearly an insect production" (p. 624). In 1880 Lambotte admitted: "Quelques auteurs croient que cette espèce (*Psilonia nivea* Fries) est une production des insectes" (p. 256).

When Saccardo placed certain of Fries' species of *Psilonia* in the genus *Volutella*, he included *V. nivea* (Fries) Sacc., with *Psilonia nivea* Fries as a synonym, edited Fries' description, and—evidently overlooking Berkeley's earlier correction (1865, 1867)—concluded with the opinion: "Sec. Cooke est opus insectorum, quod vero descriptio auctorum non confirmat" (Saccardo 1886 : p. 685). It seems evident that he referred to Fries' original description, and that he accepted it as referring to a fungus. Two years later, Cooke stated that *Volutella nivea* "is *Adelges Fagi*, according to authentic specimens"

(1888 : p. 13). Thus it can be asserted reasonably that the name *Psilonia nivea* applies, if not to the woolly beech scale itself, to an integral part of it. Cooke's statement was compiled without comment by Saccardo (1892), and was accepted by Massee (1893). *Volutella nivea* (Fries) Sacc. was included in the fungal flora of Belgium by De Wildeman and Durand (1898). As late as 1909, Lindau listed *V. nivea* as a fungus on the bark of *Fagus*. He also made two erroneous assumptions: first, in quoting Cooke's statement, he assumed that *Psilonia nivea* Fries is the product of a leaf-sucking aphid, apparently because the aphid *Phyllaphis fagi* (= *Adelges fagi*, *pro parte*) actually occurs on beech foliage, although Fries stated: "E rimis corticis Fagi" (1832 : p. 450), and Cooke likewise stated: "On bark of *Fagus*" (1888 : p. 13); second, he believed that Saccardo had actually examined and described other material of true fungal nature—a conclusion which does not follow from Saccardo's (1886) own statement. Finally, Weese (1931) evidently also accepted Cooke's claim.

In short, it is held by the present writer that the "fungus" described by Fries under the name *Psilonia nivea* is the wool-like waxy secretion of the beech scale and as such deserves discussion in a historical résumé of the nomenclature of this insect; this decision is based on (1) Cooke's statement that he examined an authentic specimen from Fries, and (2) the fact that the Libert exsiccatus specimen (1838, No. 387) examined by the present writer consists solely of the "wool" and shrivelled bodies of the woolly beech scale on *Fagus* bark. Although the fungal nature of *Psilonia nivea* was attested to—without careful examination—by Berkeley and Broome (1859), Berkeley (1860), Kickx (1867), Lambotte (1880), Saccardo (1886), De Wildeman and Durand (1898), and Lindau (1909), its insect origin was recognized by Berkeley (1865, 1867), Cooke (1871, 1888), Massee (1893), and Weese (1931).*

The first generally recognized reference to the woolly beech scale as an insect is by Baerensprung (1849), whose description is perhaps inadequate according to present standards but leaves no doubt as to the identity of the insect which he recognized as an undescribed species of the genus *Coccus* and named *C. fagi*. This was in Germany. Late the same year in Scotland M'Intosh (1849) mentioned a white, rapidly spreading fungus, giving the bark of trunk and branches the appearance of being covered with snow; his own subsequent statement leaves no doubt that this "fungus" is no other than the woolly beech scale. A few weeks later C.C. (1850 : p. 11) stated that the disease referred to in 1849 " . . . . is caused by one of the Aphides (*Aphis Fagi*), which attacks the bark of the Beech . . . . "; he neglected to mention whether this name is his own or an old one. In an editorial comment appended to C. C.'s communication, M'Intosh observed that Balfour also considered the disease to be of animal origin, "probably a species of *Coccus*" (C.C. 1850 : p. 11). A few weeks later in the same journal M'Intosh (1850 : p. 45) pointed out that "The Aphis Fagi is, we believe, always found on the

**It is hoped that this unfortunate error—whereby a secretion of an insect, being mistaken for a fungus, was given mycological status and a technical name—will not be seized upon in future by some ardent taxonomist of insects, and the name Psilonia nivea Fries held up as the one and true name for the woolly beech scale.*

foliage of the Beech, not the bark." Later the same year Hardy (1850) published a detailed and rather good description of the insect. He pointed out that it was obviously not a member of the "Aphidae," but probably one of the Coccidae, and that his description of the egg and larva corresponds with that of "certain young *Cocci*" and also with that of the related "*Lecanium* or *Chermes*." He quoted from a letter written near London by Walker: "1847 was I think the first year in which a cottony substance appeared on the trunks of Beech trees . . . . It is a *Coccus* much resembling the white bug *(C. Adonidum)* in houses, and as nearly allied to *C. Quercus* . . . . " (Hardy 1850 : p. 170). Walker wrote further that as this insect appeared to be unnoticed by authors he suggested calling it *Coccus fagi*—the very name which Baerensprung (unknown to Walker) had already employed. Two years later—apparently still unaware of Baerensprung's account—Walker (1852) published a brief description of the insect from specimens in the British Museum, under the name *Coccus fagi;* this name was accepted by Balfour and by Hardy, who wrote to Balfour: "The cottony substance sent is produced by Coccus fagi Walker . . . . " (Balfour 1863 : p. 267-8). An unsigned editorial reply to R. T. P. (1858) in the Gardeners' Chronicle stated that the insect in question—which was undoubtedly the woolly beech scale—is "the *Psylla Fagi*," without including either the origin of the name or a description of the insect. In 1870 A. M. noted that "the piece of beech bark sent is completely covered with the white excrescence of a Coccus, Adelges fagi . . . . " (p. 389). As with other such notes, neither the origin of the name nor a description of the insect was included.

Meanwhile in Germany, Ratzeburg, in the sixth edition of his "Waldverderber" (1869), had mentioned a species of Schildlaus in the genus *Coccus* discovered by R. Hartig on beech. In 1872 Kaltenbach, under the designation "? Chermes fagi n. sp.," gave notes on description and life history of an insect on beech which formed a woolly weft much like that of *Chermes corticalis* Kalt. on Weymouth pine *(Pinus strobus* L.); although Kaltenbach thus evidently assumed this insect to be an aphid, there is little doubt that he dealt with the woolly beech scale. This is indicated by the fact that his countryman Hartig (1880) discussed and figured what is unquestionably *Cryptococcus fagi* under the name "*Chermes fagi* Kltb." In previous accounts, however, Hartig referred to this insect as *Chermes fagi* Ratzeburg (Hartig 1876) and merely as *Chermes fagi* (Hartig 1878). In 1881 Altum—unaware of any previous descriptions—gave notes and sketches of the same insect and, apparently because of its superficial resemblance to species in Kaltenbach's genus Chermes (in respect to the woolly secretion), called it by the very name which Kaltenbach—unknown to Altum—had used, namely *Chermes fagi.*

Meanwhile Signoret (1873), chancing on a short description of "*Coccus fagi*" published by Hardy in 1864, for which a cottony down is mentioned, wondered if Hardy meant the "aphis du hêtre" and tentatively renamed it "*Pulvinaria ? fagi* Hardy." Having meanwhile discovered Baerensprung's (1849) and Walker's (1852) reports, but having apparently never seen a specimen of the insect, Signoret (1876) presumably reconsidered his guess of three

years previous and now guessed that this species should be placed in his genus *Pseudococcus.* A decade later Douglas (1886) reviewed the accounts of Signoret (1873, 1876), Hardy (1885 a, b), Baerensprung (1849), and Walker (1852), and concluded his remarks with the following temporary acceptance of Signoret's *Pseudococcus fagi.*

"The genus *Pseudococcus* Sign., is not exactly the same as the original *Pseudococcus,* Westwood, ... but not to argue nor to put too fine a point on the matter, the genus, in either case, may be regarded as, like Mercutio's wound, not of strictly definite dimensions, and like it also—''tis enough, 'twill serve'—for this occasion" (Douglas 1886 : p. 154). But by 1890 Douglas had reconsidered the matter, and published the following curt and incisive statement, which was destined to launch the now generally accepted new genus *Cryptococcus:* "the species *Coccus fagi,* Baerensp., hypothetically referred by Signoret to *Pseudococcus* (cf., Ent. Mo. Mag., xxiii, p. 153), has nothing to do with that genus, and as it does not accord with any other, I suggest for it a new genus, CRYPTOCOCCUS; the characters are included in those of the species, *l.c.* It may have some, but not close, affinity with *Xylococcus,* F. Löw (Verh. k. k. zool.-bot. Gesells. Wien, 1882, p. 274); at present the position of both is doubtful." (Douglas 1890 : p. 155.)

With the exception of the careless confusion by Schröder (1897) of the apple aphid (*Schizoneura lanigera* Housm.) with the woolly beech scale, and of Nüsslin's conservative use of *Coccus fagi* Baerensprung in 1905 and Barbey's in 1913, all subsequent authors, including monographers of the Coccidae, have accepted Douglas' genus and refer to the woolly beech scale as *Cryptococcus fagi* (Baerensprung).*

The foregoing annotations to the nomenclature of *Cryptococcus fagi* are summarized in the list which follows. Here names proposed by their authors as new are in bold face type; names questioned by their authors are preceded by the question mark (?). Names in brackets ([ ]) were thought by their authors to refer to a fungus. The dates in parentheses (()) refer to the year of publication (the references being cited at the end of the paper). When the authority following the colon (:) is in parentheses, he accepts the generic name given; when either authority is without parentheses, he is responsible for the generic name.

Chronological Summary of Latin Names

[**Psilonia nivea** Fries (1832)]
[*Psilonia nivea* Fries : (Libert 1838)]
[*Psilonia nivea* Fries (1849)]
Coccus fagi Baerensprung (1849)
[fungus (not named) M'Intosh (1849)]
Aphis Fagi (?) : (C.C. 1850)
Coccos sp. Balfour : (*In*, C.C. 1850, by M'Intosh)
Coccus Fagi Walker (MS.) : (Hardy 1850)
Coccus fagi Walker (1852)
Psylla Fagi (?) : (*In*, R.T.P. 1858, by ?)
[*Psilonia nivea* Fries : (Berkeley and Broome 1859)]
[*Psilonia nivea* Fries : (Berkeley 1860)]
Coccus fagi Walker : (Balfour 1863)
Psilonia nivea Fries : (Berkeley 1865)
Psilonia nivea Fries : (Berkeley 1867)
[*Psilonia nivea* Fries : (Kickx 1867)]
Coccus sp. Ratzeburg (1869)
Adelges fagi (?) : (A.M. 1870)
Psilonia nivea Fries : (Cooke 1871)
? **Chermes fagi** Kaltenbach (1872)
? **Pulvinaria fagi** (Hardy) : Signoret (1873)
? **Chermes fagi** Ratzeburg : (Hartig 1876)
Chermes fagi (?) : (Hartig 1878)
Chermes fagi Kaltenbach : (Hartig 1880)

**The parentheses denote that the generic name is another's. The botanists' custom of appending, in addition, the authority for the new accepted genus is regarded by zoologists as decidedly gauche and it is accordingly omitted with due respect by the present writer.*

[*Psilonia nivea* Fries : (Lambotte 1880)]
Chermes fagi Altum (1881)
? **Pseudococcus** *fagi* (Baerensprung) : Signoret (1876)
Pseudococcus fagi (Baerensprung) : (Douglas 1886)
[**Volutella** *nivea* (Fries) Saccardo (1886)]
Volutella nivea Saccardo : (Cooke 1888)
Cryptococcus *fagi* (Baerensprung) : Douglas (1890)
[*Volutella nivea* (Fries) Saccardo (1892)]
Volutella nivea Saccardo : (Massee 1893)
Adelges fagi (?) : (Massee 1893)
Cryptococcus fagi (Baerensprung) : (Sulc 1896)
? *Schizoneura lanigera* Housmann : (Schröder 1897)
[*Volutella nivea* (Fries) Saccardo : (De Wildeman and Durand 1898)]
Coccus fagi Baerensprung : (Nüsslin 1905)
[*Volutella nivea* (Fries) Saccardo : (Lindau 1909)]
Coccus fagi Baerensprung : (Barbey 1913)
Cryptococcus fagi (Baerensprung) : (Most others subsequent to 1900)

History and Range

In Europe

The first reference to the woolly beech scale, with which the writer is acquainted, is by Fries (1832), in which the "wool" of the insect is described as the "fungus" *Psilonia nivea;* the source of this material is not stated, but, because the same name appears in his "Summa vegetabilium Scandinaviae" (1849), the specimen may be assumed to have come from one of the Scandinavian countries. The Libert (1838) specimens were collected in the Department of Ardennes in the north of France. The first account describing the insect was by Baerensprung (1849) from the Tiergarten in Berlin. Early records from Scotland by M'Intosh (1849, 1850) state that the insect had been present for some years previous at Dalkeith Park, Preston Hall, and Moredun. The first good description was by Hardy (1850) from Scotland; he quotes Walker as writing that the insect had been first observed near London in 1847, and M'Intosh as having observed it 12 years previous (*i.e.* 1838). Other early British notes include that of C. C. (1850), regarding the outbreak in Dalkeith Park; R. T. P. (1858); Berkeley and Broome (1859) in Huntingdonshire, and Berkeley (1860, 1865, 1867); Anon. (1861) at Tunbridge Wells; Balfour (1863) at Tynninghame and Mellerstain; Foggo (1863), who stated that an unnamed insect (which Boodle and Dallimore [1911] are confident is *Cryptococcus fagi*) had been attacking beech trunks for two or three years prior to 1858 and ". . . . is pretty widely spread throughout the country . . . ." (Scotland) (Foggo 1863 : p. 344-5); A. M. (1870); Cooke (1871); Hardy (1885 a, b), reporting the insect from several localities in Berwickshire; Massee (1893); and Miss Ormerod (1898), reporting it at Burton-on-Trent and Newcastle-on-Tyne. Two years later Miss Ormerod (1900) stated that the insect was widely distributed in England, and Newstead (1900) thought that it was probably common throughout Europe wherever beech occurred. Shortly thereafter Wilks reported it from Surrey, Sussex, Kent, and Essex in the southeast of England, and feared that "the Beech is doomed all over the country" (Wilks 1901-1902 : p. 599). In his excellent account of the life history and description of the insect, Newstead (1903) characterized the woolly beech scale as one of the commonest native coccids, generally and very widely distributed throughout the British Isles, and mentioned specifically 24 counties or regions in various parts of England, Scotland, Wales, and one in Ireland (Dublin) where it was known to occur. In the same year Carpenter (1903) reported it from the Botanic

Gardens of the University of Dublin, and from Rostrevor in Ireland. Subsequent substantiating notes for the British Isles include those of Brotherston (1904), Theobald (1904, 1905, 1906, 1907, 1908, 1909, 1911, 1912, 1913), Boulger (1907), Gillanders (1908), MacDougall (1908), Webster (1908), the Board of Agriculture (Anon. 1910, 1911), Green (1915), Warburton (1918, 1922), and the Forestry Commission (1926). Worthy of special comment are the detailed observations of Boodle and Dallimore (1911 : p. 339), and their conclusion: "There can be no doubt that the Coccus is widely distributed about the country at the present time, as it has been for the last 50 years. It is also equally evident that it has, on the whole, increased in quantity during the last 15 years . . . ."

Meanwhile, in Germany Hartig had been impressed by the abundance of the insect at Eberswalde (Prussia) since 1867, and in 1880 reported it as quite general throughout Germany. Altum (1881) noted its especial abundance in the western provinces, notably Westfalen. Judeich and Nitsche (1895), in their eighth edition of Ratzeburg's textbook, stated that the insect occurred throughout central Europe and England. Subsequent notes or papers by Schröder (1897), Reh (1903), Lindau (1909), Bertelsmann (1913 a, b), D. in E. (1914), Rhumbler (1914 a, b, 1915, 1922, 1931), Eckstein (1920), Sachtleben and Pape (1922), and others, indicate that the insect is still widespread in Germany and attracting the attention of entomologists and foresters.

For the remainder of Europe, it has been reported from Belgium by Kickx (1867) in Flanders, Lambotte (1880), De Wildeman and Durand (1898), Huberty (according to Judeich and Nitsche 1895), and in the Forêt de Soignes by the Great Britain Board of Agriculture (Anon. 1910), and by Vayssière (1926), who also examined many specimens in the Limbourg belge. French records include those of Libert (1838) from Ardennes; Marchal (1908), who found the insect in small quantity on old beeches in the Forêt de Montmorency near St. Leu (Seine-et-Oise) but believed it to be rather rare in the environs of Paris; Barbey (1913); P. L. (1916); and Vayssière (1926), who stated that Marchal in 1907, and P. Lesne had also seen the insect in the Bois de Meudon, but who himself had never found it in the magnificent beechwoods of Normandie, nor in the Forêt de Rambouillet, nor in the beech stands of the French Alps. It was reported from Switzerland by Marchal (1908) at Lausanne, and (in addition to other countries) by MacGillivray (1921). It was reported from Czechoslovakia as occurring in several localities in Bohemia (Sulc 1896); from Holland as spreading slowly (Anon. 1921); from southern Sweden as occurring comparatively rarely (Trägårdh 1921); and from Denmark (in addition to other countries) by Graham (1927), although the present writer has discovered no specific references to its occurrence there.

Concerning the European range of *Cryptococcus fagi*, Vayssière (1926) pointed out that although beech occurs in all of the temperate portions of Europe, Asia, America, and Australia, the woolly beech scale has been re-

ported thus far only in the cooler parts of the European beech range. He regards the insect as a northern coccid, which appears to be limited in its southward distribution in Europe by latitude 49 degrees north. Although in North America the insect already exists as far south as latitude 42 degrees north, it is quite possible that this is permitted by the cooler climate of the western shores of the Atlantic and that future distribution of the insect into the beech stands of the central and southern Appalachian uplands may be limited by their warmer climate.

In North America

In America there are no records at hand prior to 1914, other than its recognition as a European insect by Cockerell (1899) and Fernald (1903). In 1914 Braucher published the following correspondence: (1) from a letter regarding this insect, written October 2, 1913, by L. G. Vair: "The trouble is noticeable all through the woods in the vicinity of Halifax, N.S. . . . ."; (2) from a letter written November 6, 1913, by Dr. L. O. Howard: "I have referred this material to Mr. Sanford, who is of the opinion that it is the European felt scale (*Cryptococcus fagi* Baerens.). So far as I am aware, this is the first report of the occurrence of this coccid in North America . . . . Doubtless it has been imported on European stock, and measures should be taken to prevent its becoming widely distributed" (Braucher 1914 : p. 14). Shortly after the appearance of Braucher's note, Hewitt (1914), the Dominion Entomologist for Canada at that time, published a note correcting Braucher's impression that the insect had been found for the first time in America. He reported having received specimens in 1911 from Bedford and Halifax, that he had found the insect on both ornamental and forest beech in the vicinity of Halifax where it had apparently existed for a number of years, and that the superintendent of the Public Gardens in Halifax had known of the presence of the insect for at least 20 years, where he had been spraying the purple-leaved variety of beech. He was of the opinion that the insect might have been introduced into Canada a number of years previously on ornamental beeches from Europe. It appears from these two accounts that the woolly beech scale was present in Halifax probably as early as 1890, that it occurred on imported specimens of the European beech (*Fagus sylvatica* L.) at the outset and may well have been introduced with such specimens, and that by 1914 it had become generally established in the stands of American beech (*Fagus grandifolia* Ehrh.) in the forests about Halifax.

In the following years the insect apparently spread steadily from its assumed point of origin at Halifax. By 1926 Hutchings reported it as established not only in Nova Scotia but also in Prince Edward Island; and Motherwell (1926) mentioned it as established in parts of the Maritime Provinces. By this time reports of the injury and death which attended infestation were becoming frequent and widespread, with the result that a preliminary investigation was made by the Dominion Entomological Branch and the Nova Scotia Department of Lands and Forests, which showed the insect to be present in every county in the province, but not yet in the adjoining province

of New Brunswick (Graham 1927). In 1929 Gorham observed the insect to be abundant in stands of beech in Prince County, Prince Edward Island, and late the same year Gorham, Walker, and Simpson (1929) reported it for the first time in the part of New Brunswick which adjoins Nova Scotia, stating that it had been first noted in 1927 near Dorchester in Westmoreland County and in Albert County, and that at the time of writing (1929) it was present in most of the beech areas of both counties. Subsequent notes on its occurrence in these areas were made by Swaine (1930), Ehrlich (1931, 1932 b), Balch (1932), and Balch and Simpson (1933). Observations were continued by members of the Federal Entomological Branch Laboratory (Fredericton, N.B.), with the result that by the autumn of 1932 the insect was found in a number of beech stands in the southern part of New Brunswick, not only in Westmoreland and Albert Counties, but also in Queens and Charlotte Counties (Fig. 1) (Ehrlich 1933 b).

In the United States the woolly beech scale was apparently first recognized in November, 1929, upon its discovery in Boston by the present writer (Ehrlich 1932 a) on ornamental beech in the Arnold Arboretum and in certain of the park lands. Both the appearance of the infestation and the word of a park employee indicated that the attack was about 10 years old in certain places. Further search with the assistance of the Boston Park Department lead to the discovery of infestation in a number of points throughout the Metropolitan District. When in the spring of 1931 the United States Bureau of Entomology was informed of the presence of this insect, through chancing on a control experiment being prosecuted under the direction of the writer in the Middlesex Fells, members of the staff of the Melrose Highlands Laboratory joined in the search. Several additional outbreaks were discovered, and by autumn of 1932 infestations were known to exist in Boston (Suffolk County), Brookline (Norfolk County), and the neighboring towns of Gloucester, Essex, Manchester, Beverly (Essex County), Stoneham, Cambridge, Belmont, Watertown, Newton (Middlesex County), and Milton (Norfolk County), but none beyond, although search was made by members of the staff of the Melrose Highlands Laboratory in many areas on all sides of Boston as well as points in Rhode Island and New Hampshire. During 1931 a young outbreak was found in the town of Liberty, Maine, by members of the Melrose Highlands Laboratory. Further scouting in Maine by the State Entomologist during 1932 lead to the discovery of additional outbreaks in surrounding towns and an older infestation in eastern Maine adjacent to the New Brunswick border. By the winter of 1932-1933 the insect had been found in the following Maine towns: Robinston, Perry, Baring, Meddybemps, Charlotte, Pembroke, Dennysville (Robinson), Northfield, and Whitneyville in Washington County (eastern Maine); Montville, Palermo, and Liberty in Waldo County, Somerville in Lincoln County, and Washington in Knox County (south central Maine) (Peirson 1932, 1933 a, b).

The entire known American range is indicated approximately on the sketch map (Fig. 1). In addition to the note reporting the first discovery of

the insect in the United States (Ehrlich 1932 a), other notices dealing with outbreaks in New England were published by Collins (1931, 1932), Schaffner (1931), Peirson (1932, 1933 a, b), Hayden (1932), Beattie (1933), Felt (1933), and Ehrlich (1933 a, b).

It is not known how or when the insect made its first appearance in the United States. That it was introduced is most probable. It may have come to Massachusetts from the Maritime Provinces, with which there is much intercourse both by land and by sea, either on nursery stock or as bits of wool containing eggs and crawlers, clinging to anything and being disseminated by the wind on arrival. Or it may have been introduced directly from Europe on stock of *Fagus sylvatica*, which is commonly planted in parks and estates throughout the now infested area. So far as the writer is aware, there is no direct evidence in support of either alternative. The outbreak in eastern Maine may well be a spreading of the infestation in the Maritime Provinces. Whether the insect now occurs elsewhere, other than in south central Maine, is not yet known; but if not, it is difficult to understand how or why this particular area, which is sparsely settled and not traversed by trunk railroads or important highways, should have been the only point attacked.

Description

Although *Cryptococcus fagi* was first reported as an insect by Baerensprung in 1849, and partially described by Hardy in 1850, it was not until 1886 that Douglas published a detailed description. Other careful observations were reported by Sulc (1896) and Newstead (1903). Certain of the early descriptions are included in the present account for their historical interest, beginning with Fries' (1832) "*Psilonia nivea*," which is believed to be the woolly secretion of this insect.

"*P. nivea*, caespitibus compactis laneis candidis, floccis ramosis intorto-intricatis.

"Caespites erumpentes, rotundi, 1—2 lin. lati, lineam alti, compacti, licet flocci omnino discreti sint, rotundato-irregulares, confluentes. Flocci crassiusculi, non pellucidi, sed transparentes tantum, ramis patentibus instructi, contigui, intorte et implexi, apice attenuati. Sporidia (potius *Gonidia)* minuta, globosa, tarde diffluentia, sed subinde immixta vidi sporidia cylindrica, curva, ut ad Menisporas pertineret. *E rimis corticis Fagi erumpit hieme.*" (Fries 1832 : p. 450.)

The "sporidia" which Fries saw in his surface examination of the material which he thought to be erumpent from the sub-phellar bark do not, in the present writer's judgment, cast doubt on the belief that the material was in truth the "wool" of *Cryptococcus fagi;* the spores of any one of a number of Mucedinaceous fungi may well have been present and been misinterpreted as belonging to the material under observation.

The first formal description of the insect is the following brief note by Baerensprung (1849 : p. 174):

"♂ ?

"*♀ lutea, ovata, abdominis apice hirsuta; capite minuto, antennis brevibus crassis. Long. ¾ lin.*" *This is supplemented with notes summarized as follows. Females enclosed in a thick weft, produced especially by the hind part of* the thick, soft, sulphur-yellow animals. Antennae short and thick, next to the black eye-spots. The short legs almost completely retracted into the fleshy body. Larvae of same elliptical shape, with two red eyepoints, five-membered antennae with bristles at their ends. Last member of hind leg provided with two pairs of papillae, a smaller inner one and a larger outer one, with a few bristles between.

Walker's (1852) description of his proposed new species, *Coccus fagi*, was quite as laconic:

"Flava, elliptica, albo-farinosa.

"Yellow, elliptical, covered with white powder. Length of the body 2 lines. England." The "2 lines" is obviously an error since a line equals $\frac{1}{12}$ inch, and the largest females never attain anything like such size.

The "woolly" secretion of the insect was subjected to chemical analysis and reported to be a new wax, very close to China wax, having a melting point of about 80° C., and the following composition: C, 81.39; H, 13.58; and O, 5.03% (Anon. 1884). Various tests on this material were reported by Hardy (1850).

The first careful description of the insect was given by Douglas (1886). Its main features are summarized in Tables VII and IX. A decade later it was reported (Judeich and Nitsche 1895) that recent observations by Nitsche showed the description of the legs of the adult as given by Baerensprung and by Douglas to be in error; Nitsche stated that the adult female does not have reduced legs embedded in the body, but has no legs at all, only vestiges marking their earlier position. More recent observations have shown that Nitsche was correct. Sulc (1896) redescribed the insect, giving notes on the diagnostically important anal characters (see Tables VIII and IX), and publishing the first accurate drawings. In his "Monograph of the Coccidae of the British Isles," Newstead (1903) gave an excellent illustrated account of *Cryptococcus fagi*, the salient points of which are included in Tables VII, VIII, and IX. When Rhumbler (1915) reported his studies of this insect, he gave measurements

TABLE VII

COMPARISON OF DESCRIPTIONS OF EGG, *Cryptococcus fagi*

	Douglas	Judeich and Nitsche	Newstead
Color	Pale yellow	—	Lemon-yellow
Shape	Oval	—	—
Size	Large	Approx. 0.6 mm.	Very large in proportion to size of insect
Number laid at sitting	7–8	—	4 or 5 in strings attached end to end

of the first two nymphal forms and the adult, which are presented in Tables VIII and IX.

Illustrations of the insect appear in Fig. 10. Hartig's (1880) sketch of the crawler (Fig. 10 A), although lacking in detail, gives an excellent general impression. A more detailed original camera-lucida drawing (Fig. 10 B) of dorsal (right half) and ventral (left half) aspects shows the position of spines and hairs, and the structure of legs and antennae. More highly magnified original drawings from ventral and dorsal aspects of the anal

TABLE VIII

COMPARISON OF DESCRIPTIONS OF CRAWLER, *Cryptococcus fagi*

	Sulc	Newstead	Rhumbler Form I	Rhumbler Form II
Color	Reddish yellow	Lemon-yellow	—	—
Shape	Elliptical	Elongate ovate	Slim, turnip-shaped	Thicker set
Size	—	—	0.24–0.33 mm.	0.35–0.38 mm.
Body length / Leg length	—	—	3/1	4/1
Eyes	Distinct, reddish brown	Large	—	—
Antennae	5-segmented 2nd > basal, 5th, > 3rd, 4th; 2 small hairs on each segment; 2 short obtuse and 2 long fine hairs on 5th segment	5-segmented 2 > 5 > 1 > 3 and 4	5-segmented	5-segmented
Rostrum	Mentum dimerous, with hairs	Large, mentum uniarticulate	—	—
Stylets	—	Exceeding length of body	—	—
Legs	Strong; coxa large; trochanter wedge-shaped, with rather long hair; femur massive, as long as tibia and tarsus together; 2 pairs digitules on tarsus; a hair in incision proximad of inferior pair; terminated distally by simple untoothed claw equal in length to tarsus	Short; stout; tibiotarsal segments short and equally divided; claw long; digitules to claw and tarsi ordinary	Present	Present
Abdomen	Distinctly segmented; 2 hairs on side of each segment	—	—	—
Anal segment	2 pairs of tubercles with strong conical spines on apex; 1 long bristle on base of each single inferior tubercle (ventrally); inwards from it a small simple hair	Slightly truncate, lobes faintly projecting but extremely minute, each with single spine; similar small spiniferous lobule on ventral surface; single long slender hair arising from base of each lobe on ventral surface	—	—
Anus	Orifice oval, with superior pair and inferior pair of hairs	—	—	—

TABLE IX

Comparison of descriptions of adult female, *Cryptococcus fagi*

	Douglas	Sulc	Newstead
Color	Sulphur-yellow	Canary-yellow	Lemon-yellow
Form	Short rounded-oval, nearly circular, very convex above and below, almost globose	Elliptical, almost sub-circular	Hemispherical, convex above, comparatively flat beneath, anal extremity slightly curved ventrally
Length	Barely ½ line long	0.44 × 0.47 mm.	0.75–1.00 mm. (0.5–0.8 mm. : Rhumbler)
Hairs, spines	Finest pubescence above, no projections at sides or end except pale setaceous hairs on end	—	Minute spines numerous
Segments	All determinable, the junction of their ventral and dorsal rings forming a continuous thickening	Segmentation of pre-abdominal part indiscernible	Segmentation faintly indicated
Eyes	2, on ventral anterior margin of head, blackish, angular, rounded in front, extending obliquely inward and downward to long fine point	Simple, reddish brown, on anterior periphery	—
Rostrum	Short, appressed, covered, with free end turned at right angle, brown, tubular	Mentum dimerous, with 2 long and 6 short hairs	Reddish brown, mentum uniarticulate
Stylets	Extremely fine blackish "seta," 3 times length of body	—	Nearly 2 × length of body
Antennae	Short, thick, apparently of 3 segments only, concolorous	Atrophied, only a stump with 2–4 long hairs	Reddish brown, rudimentary, of 3 segments, generally 2 short stout spines on 2nd segment, shorter spine on 1st segment
Legs	Short, embedded in the fat body, concolorous, difficult to see	1st and 2nd pairs absent, only saccate stumps of 3rd pair present	Reddish brown, 1st and 2nd pairs obsolete, 3rd pair represented by minute tuberculate projections
Wax glands	—	On whole surface of body without order, especially on abdomen	Numerous, minute tubular and large circular, latter forming distinct bands on abdominal segments above
Anus	—	Elliptical, with thickened margin, 2 hairs anteriorly and 2 posteriorly, on each side 3 strong spines	Anal lobes obsolete, position indicated by single large spine and short stiff hair; 2 similar hairs anterior to anal orifice on ventral surface; anal orifice with 4 spines in 2 pairs, 1 anterior, 1 posterior; between anterior are several circular glands; small inner chitinous ring

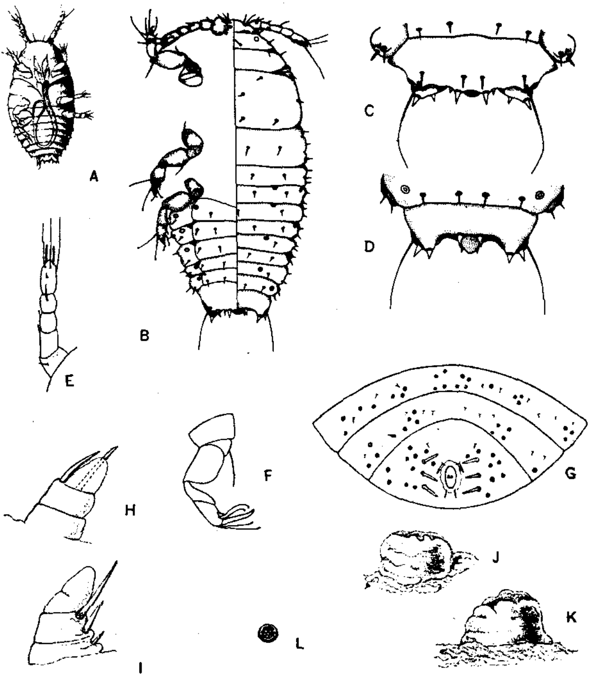

FIG. 10. *A. Crawler of Cryptococcus fagi (from Hartig 1880). B. Crawler; right half, dorsal; left half, ventral (original). C. Anal region of crawler, ventral aspect (original). D. Anal region of crawler, dorsal aspect (original). E. Antenna of crawler (from Sulc 1896). F. Leg of crawler (from Sulc). G. Anal region of adult (from Sulc). H. I. Vestigial antennae of adult (from Newstead 1903). J. K. Vestiges of third pair of legs of adult (from Newstead). L. Wax plate of adult (from Sulc).*

region of the crawler (Fig. 10 C and D) show the exact locations of the diagnostically important anal spines, hairs, and bristles. Sulc's drawings of antenna and leg appear in Figs. 10 E and F, respectively. The anal region of the adult, as figured by Sulc (Fig. 10 G) is somewhat diagrammatic but correct in the number and location of spines. The vestigial antennae of the adult and the vestiges of the third pair of legs (from Newstead) appear in Fig. 10 H, I, and J, K, respectively. A wax plate from Sulc is represented in Fig. 10 L.

In brief, this insect is a typical coccid, with oviparity, motile first-instar nymph, degenerate parthenogenetic adult female, and male unknown. The "scale" of this species consists of a loose mass of curled white threads which form the ovisac and a fluffy wool-like canopy over the colony. The diagnostic characters of the first instar nymph or crawler are two pairs of setiferous anal lobes, the ventral pair bearing each a long slender bristle, two pairs of anal spines, three pairs of strong typical legs, and five-segmented antennae. The distinguishing features of the adult female are vestigial three-segmented antennae, absence of the first two pairs of legs and tuberculate vestiges of the third pair, two trimerous sets of lateral spines flanking the anus, and an anal ring bearing an anterior and a posterior pair of spines.

Life History and Habits

Scattered notes on the life history of *Cryptococcus fagi* occur in the early literature. Baerensprung (1849) in Germany reported finding eggs and nymphs in December, but no adults. Kaltenbach (1872) found eggs and dead females in winter, nymphs in spring, and again eggs and nymphs in summer. Kessler (1884) reported eggs in winter, eggs and nymphs in spring, eggs, nymphs, and pregnant females in summer. Douglas (1886) in England observed eggs and crawlers in winter, females at the end of May, ovipositing females, eggs, and nymphs in the middle of July. Nitsche (1895) in Germany found eggs and crawlers in winter, eggs and nymphs in summer, and nymphs in autumn. Miss Ormerod (1900) stated that in England eggs were laid in June and that she found crawlers on July 4. Newstead (1900) stated that the crawlers hatch in September. The first careful observations on the life history of this insect were made in England by Newstead (1903). His account follows.

"The eggs are laid at the beginning of July, and the larvae hatch in the autumn, and apparently also in the spring. On hatching from the egg the majority of the larvae work their way under the bodies of their dying or dead parents, taking up their positions by preference in the deepest parts of the fissures in the bark, where they remain for the rest of their lives, pumping up the juices of the tree. Each individual protects or covers its body with secretion, which adds to that already secreted above them by their parents; thus the secretion gradually thickens and spreads over the tree trunk, eventually forming a more or less continuous mass, often attaining a great thickness . . . . In such masses one finds the remains of many generations of females. When an insect takes up a separate abode the secretion forms a complete and approximately round sac, such sacs being commonly met with on newly infested trees, or on the branches just above the trunk of a badly infested tree. It is on the trunks of the very old trees (50 to 100 years) that the insects are found in the greatest numbers, and they probably represent the survivors of many generations which have lived, cycle upon cycle, on the same tree. The larvae do not appear to be of a wandering habit, and comparatively few seek the fresh untenanted bark; the main branches, as a rule, are not attacked to the same extent as the main trunk. Those larvae which

wander over the trees are liable to be borne away by the wind or by birds to other trees, and this is probably the means by which fresh colonies are started." (Newstead 1903 : p. 219-220).

Simultaneously, Reh (1903) reported observations on the life history of this insect in Germany. His notes include winter observations of crawlers in November, old dead females and eggs in December, and nymphs in February; spring observations of nymphs on April 8, and young females on April 14, and May 21; summer observations of nymphs and females in June and in August; and autumn observations of old females, eggs, and young nymphs in September, and dead females and nymphs in October.

The next detailed studies to be reported were made in Germany by Rhumbler (1914 a, b, 1915), some of whose observations follow. The insect hibernates on the bark as a wool-clothed nymph, either as Form I, in which case transformation to Form II takes place in February, or as Form II. Between April and July the Form II nymphs moult and develop into adults. Oviposition begins in the first week of June and continues to the end of October, the adults remaining fixed throughout this period in their ovisacs composed of the curly threaded wax secretion which covers them with a fluffy white down; the act is periodic, each female laying several batches of eggs, each batch comprising not more than seven or eight eggs. The duration of the incubation period varies inversely with temperature; at the relatively uniform temperatures of the laboratory, eggs under observation hatched in 25 or 26 days during August and September; out of doors, 45 to 50 days were required during September and October. The Form I nymphs are active crawlers; although certain individuals never leave the ovisac but insert their stylets into the bark and begin feeding in the maternal nest, the majority roam over the bark, primarily upwards and towards the light, probing the bark here and there as they wander. The speed and skill of their locomotion increase with temperature; they have been observed on a warm afternoon to travel a vertical distance in the sunshine of more than two metres. Eventually they settle down, insert their proboscal setae (stylets) into the bark, and remain fixed in position thereafter. Secretion of "wool" begins rarely while they are still crawling, usually promptly after settling, and occasionally not until four weeks later. These crawlers then overwinter *in situ*, some of them changing first into Form II nymphs. The mothers die in the late autumn. The life cycle is thus annual.

In a subsequent account Rhumbler (1922) contributed the following additions or changes. The Form I nymphs occur from July to December and are present abundantly in September and October. Their crawling is interrupted at night and while the bark is wet. Following cessation of crawling, the change from Form I to Form II is one of growing and compacting of the body which retains the now useless and relatively small legs. The Form II nymphs moult between April and July and metamorphose into elongate, legless, second-instar nymphs. These nymphs moult once more and develop into egg-laying parthenogenetic females. When the latter die in late autumn

they shrink, darken in color, and are frequently inhabited by a species of *Cladosporium* which converts the outer wool into a brown weft covering the fresh white wool and younger generation beneath.

The essential points in the foregoing account were repeated by Eckstein (1920) and Reichling (1920) in Germany and by Anon. (1921) in Holland. Trägårdh (1921) found that in southern Sweden eggs occur in August and the crawlers appear later in the autumn.

In Nova Scotia Graham (1927) found "the young scale stage" on June 17, 1926; this changed to the adult during the remainder of June and all of July. Oviposition was first observed on July 22 and hatching on August 10, continuing well into September. The present writer's summer observations in Nova Scotia and southern New Brunswick show that by June the adult stage has been reached and that pregnancy is visible by the latter part of the month. The date of first oviposition varies from year to year, but occurs in the first or second week of July. Hatching may begin in a few cases by the end of July but is not common until about August 10. In Boston, oviposition and hatching begin somewhat earlier than in the Maritime Provinces; the Form I nymphs cease crawling at approximately the middle of September and by October all crawlers still alive have settled in crevices and begun secreting "wool." The species overwinters as Form I and Form II nymphs; eggs which have not hatched by September are lost.

The foregoing notes on the life cycle of *Cryptococcus fagi* may be summarized in tabular form, as in Table X.

TABLE X

SUMMARY OF NOTES ON SALIENT POINTS IN THE LIFE CYCLE OF *Cryptococcus fagi*

	Germany Holland	England	Sweden	Maritime Prov. New England
Overwinters as	Eggs and nymphs	Eggs and nymphs	?	Nymphs
Oviposition begins	Early June	June (Ormerod) Early July (Newstead) Middle of July (Douglas)	August	Early July
Hatching begins	Early July (Rhumbler) September (Reh)	September and spring (Newstead) Early July (Ormerod)	Autumn	Early August
Crawling ceases	During October	?	?	During September

Oviposition was observed by Kessler (1884) to consist in the laying at one sitting of four or five eggs in horizontal or vertical strings, followed by the addition of another egg to the string on the next day.

Although Rhumbler's statement that the larvae crawl upwards predominantly is correct, this is far from invariable; Rhumbler himself reported

catching them in great numbers on glue-coated slides placed on the forest floor; however, it is probable that many of these fell or were washed or blown from the trees. The numbers that crawl during favorable weather are enormous; fresh bands of tar painted around trunks of beech in the forenoon were bordered by afternoon with a quantity of larvae so great that a fine yellow margin could be detected at a distance of several yards from the tree; and this margin was almost as dense on the upper side of the band as on the lower, indicating that with ample warmth and sunshine the orientation is not rigid. Although great numbers of the wandering larvae are blown away, washed down by rain, or killed by exposure, many eventually settle in sheltered places suitable for feeding; such places include lenticular crevices and other cracks or wounds, especially on the more shaded and lee side of the trunk, abaxillary angles of the lower branches, the undersides of branches, and areas under thalli of mosses and foliose lichens epiphytic on the bark.

All stages of the species feed by forcibly inserting their slender powerful stylets (modified maxillae and mandibles) into the bark and pumping and sucking in the manner characteristic of plant-inhabiting insects with sucking mouthparts. The strength with which the stylets are directed is attested to by the fact that when the observer forcibly raises an individual so that the stylets are partly withdrawn from the bark, the insect is frequently seen to wave its body in the air, pivoted only on the stylets. Their mobility can be seen by raising an individual from the bark, turning it so that its back rests on the substratum, and watching the wavings and bendings of the raised stylets; this flexibility is also indicated by the often tortuous courses and bifurcations of the stylet sheaths when observed in sections of the bark, which show that in the course of prolonged feeding in one area on the bark the insect probes in all directions into the tissues within reach of its stylets, bending them at any point in their length, as demanded by the tissues encountered.

Dissemination and Distribution

Local spread of *Cryptococcus fagi* takes place during the period in its life cycle when it is capable of becoming established on a fresh substratum, that is, as eggs and as Form I nymphs; older stages are transported by various agencies but, being legless, are unable to seek favorable nesting places and die of exposure and starvation. Since there are no winged forms, dissemination is effected by the wandering of the crawlers (Form I nymphs) and by agencies which carry eggs and nymphs.

The crawlers are active and have been found capable of travelling a distance of two metres on a sunny afternoon (Rhumbler 1915). Although Newstead believed that "the larvae do not appear to be of a wandering habit, and comparatively few seek the fresh untenanted bark" (1903 : p. 320), this is hardly borne out by the observations of Rhumbler and of the present writer. The numbers on the move during a favorable day are enormous in a well infested beech stand; Eckstein (1920) counted over two million individuals on a single square metre of bark surface. Crawling is the principal means

of spread on individual trees and to a less extent to adjacent trees. The latter is indicated by the fact that Rhumbler (1915) regularly caught crawlers on glue-coated slides placed on the ground (although it is not unlikely that some of those caught had been blown from infested trunks), and by the present writer's repeated observation that in a recently infested stand the first attacks on nearby trees usually begin at the butt and proceed upwards. Rhumbler himself believed crawling to be of no importance whatever in initiating attacks on uninfested trees.

The leading agencies which have been assumed to carry eggs and nymphs are wind and birds. Both were suggested by Newstead (1903) and Bertelsmann (1913 a); Rhumbler (1915) suspended glue-covered slides in the air and caught great numbers of nymphs, far more than on the terrestrial slides. Similar tests were made in Nova Scotia by Graham (1927), who caught eggs and naked nymphs at maximum distances of over 300 yards from their source. The present writer also frequently trapped naked nymphs on slides set to catch fungal spores. Many authors have assumed that birds carry the eggs and nymphs and in this way play a role in local dissemination and in long-range distribution. Newstead (1903) thought that birds were probably a means of starting fresh colonies; Bertelsmann (1913 a) listed not only birds seeking insects on the trunk, especially woodpeckers, tree creepers, and nut peckers (?), but also squirrels; Reichling (1920) believed that squirrels, woodpeckers, and titmice were not insignificant carriers; Eckstein (1920) mentioned squirrels, woodpeckers, nuthatches, and tree creepers as possible minor carriers. Mammals such as porcupines, moose, deer, and bear, whose gnawings are occasionally encountered on beech in the Maritime Provinces, may also act as local agents of dispersal. So far as the present writer is aware, no critical observations or experiments devised to test these assumptions have been performed.

The means of long-range dissemination can only be conjectured. Transportation of infested beech stock is doubtless occasionally a means and was probably the source of the first Nova Scotian outbreaks. Wind can undoubtedly carry eggs and nymphs for many miles and lead to the establishment of infestations in distant areas; a limitation would be the vitality of the insect, regarding which nothing definite is known; wind seems the only likely means of bringing inoculum to so isolated and distant an area as that in south central Maine. Man and his vehicles of transportation may also carry bits of wool or naked eggs and nymphs, and scatter them occasionally on suitable substrata. In short, effective spread of the insect takes place by means of eggs and Form I nymphs, which, in most countries other than England where the insect has been studied, is during the months of June to November. Local dissemination is by crawling and wind, and possibly by birds and mammals. The means of long-range distribution are not known with certainty, but may include wind, transportation of nursery stock, and chance carriage on ships, trains, and automobiles.

Factors Influencing Infestation

It is characteristic of infestations of *Cryptococcus fagi* that when they become established in a forest area, scarcely a beech remains free of attack, practically every tree harboring at least a few of the insects, irrespective of size and vigor of the tree, of proportion of beech to other species in the stand, of local nature of the stand, and of physiographic factors. In spite of this total infestation, differences in severity of the infestation are regularly apparent, varying with a number of conditions. Those which have been suspected of influencing severity are *(a)* the tree factors—health and size of the tree, nature of the bark, amount of lichens and mosses, position in stand, and those determining which side of the trunk is most heavily infested; *(b)* the stand factors—density of stand, position of stand, and soil; *(c)* climate.

Tree factors

Health of tree. As early as 1849 M'Intosh pointed out that old and "sickly" trees are most heavily infested; but apparently his observations were none too extensive, since Balfour (1863) claimed that only healthy trees are attacked, a view also expressed by German foresters (Piller, in Hartig 1880; Bertelsmann 1913 a). Webster (1908) believed that the severity of infestation is independent of the health of the tree. Rhumbler (1914) advanced the opinion that in an area already exposed to infestation, a marked outbreak consists merely in an increase of the infestation due to injury or disease of the more heavily attacked trees; in support of this view he pointed to the generally recognized fact that certain obviously healthy trees remain free of the insect while adjacent individuals support an increasingly dense coating. A few years later Rhumbler (1922) stated that a slime-flux infection of unknown origin is the initial disturber of the healthy tree and that its activity favors a heavy infestation by the woolly beech scale. The present writer's observations lead to no certain conviction that the original health or vigor of the tree play any part in the building up of a heavy infestation. In both the Maritime Provinces and New England slime-flux or other infections associated with this disease and subsequent *Scolytid*-beetle invasions develop only after *Cryptococcus* has been present for some time. Possible decreases in the vigor of the tree due to partial girdling by porcupines and extensive leaf injury due to *Tortricid* larvae have no appreciable influence on infestation.

Both Reichling (1920) and Eckstein (1920) pointed out that healthy and vigorous trees which are not infected subsequent to *Cryptococcus* attack frequently lose their insect inhabitants and remain uninfested for long periods. This condition has been observed in several places by the present writer; it is believed to be the result of an enormous regional increase in *Cryptococcus* nymphs due to favorable climatic conditions, and the consequent infestation of trees which under climatic conditions less propitious to the insect could not offer adequate shelter; if such trees remain uninfected, a season unfavorable to the insect causes the death of those which are not adequately protected.

Size of tree. Whether or not a beech becomes infested is not influenced by size or age of tree. This has been recognized from the start. M'Intosh (1849) reported the insect on all trees observed over 11 years old. Ricker (Hartig 1880) stated that infestation is independent of crown class. Boulger (1907), Webster (1908), Bertelsmann (1913 a), Eckstein (1920), and Reichling (1920) reported trees of all ages infested; Rhumbler (1915) examined systematically over 1000 beeches over two centimetres (¾ inch) in diameter in two districts of Münden and found not a single tree uninfested. The present writer's general observations in the Maritime Provinces and New England, and systematic records of more than 1000 tagged trees in permanent sample plots examined three times and of more than 4000 beech in temporary sample plots substantiate the foregoing statements. The insect was commonly found in infested stands on saplings under 4.5 ft. in height. The amount of infestation on individual trees was found, however, to vary considerably with size of tree. Small saplings usually harbor a very few small colonies, and trees of small diameter rarely support as dense a mass of insects as the larger individuals; but trees over approximately 16 inches in diameter are frequently less heavily infested than smaller ones. The writer believes these differences to be due to other factors associated with size of tree, such as nature of bark, and abundance of lichens and mosses.

Nature of bark. The characteristics of the bark which may be presumed to affect the size and number of colonies are of two kinds—those limiting the ability of the insect to puncture the bark and gain nourishment, and those determining its ability to survive unfavorable meteorological conditions. The former include depth of the cork layer, proportion of sclerenchyma to parenchyma in the tissues within reach of the stylets, hardness as influenced by moisture, nature of the shelter afforded by lenticular crevices, foliose lichens, and mosses. The effect of proportion of sclerenchyma to parenchyma has been studied for certain other plant-sucking insects, but has not yet been investigated for *Cryptococcus fagi*. Hartig (1876, 1878) pointed out that *Cryptococcus* colonies develop most abundantly on areas of young callus bark where the cork layer is thin (and where there is as yet little sclerenchyma), such as on the edges of cankers produced by frost and by *Nectria ditissima*. Rhumbler (1915) believed the nature of the bark to be a minor factor, but that the insect avoids exceedingly soft (?) and corky bark, and favors thin secondary bark such as callus over cankers and wounds produced by the antlers of deer; neither absolute nor relative thickness of bark was believed to affect infestation. Bertelsmann (1913 a) observed attack to be light on smooth, hard, shiny steel-gray bark and heavy on soft bark; Eckstein (1920), on the contrary, claimed that the latter type is more heavily attacked, while Reichling (1920) believed that soft bark is favored. Bertelsmann pointed out that bark while moist is softer than when it dries and that therefore bark in moister situations is more heavily infested.

Since the smooth unbroken bark offers no shelter to the insect against driving rains, strong winds, and hot sun, the insects are usually more numerous

in places where some protection against these elements exists. Wounds, cracks, lenticular crevices, mosses, and foliose lichens all provide such shelter. In addition, all retain moisture following rains for longer periods than the smooth exposed surfaces of the bark and therefore maintain the bark in a condition better suited to penetration by the stylets. The role of lichens was recognized by Hartig (1876), by Bertelsmann (1913 a) who pointed out that the insects avoid trees from which *Limax arborum* has removed the lichens and *Protococcus pluvialis*, and by Reichling (1920). The present writer found the bark beneath lichens which abound on beech in the Maritime Provinces (especially species of *Parmelia*) to be the first attacked; in fact, many newly infested trees which showed little or no sign of the insect at first glance were found to harbor considerable numbers under the lichen thalli. This condition was most pronounced in somewhat open stands where the exposed bark offered none too favorable a substratum.

Side of tree. It has often been thought that certain sides of the trunk should be more heavily infested than others. Kaltenbach (1872) detected no differences in infestation on the various sides; Ricker (Hartig 1880) saw no differences within the stand but observed infestation at the edges of stands on the eastern side only, which was to the lee of prevailing winds; similarly Boulger (1907), the Forestry Commission (1926), and Graham (1927) found infestation heaviest on the sheltered side of exposed trees; Bertelsmann (1913 a) found that in his district the east and north sides were preferred, and attributed this to more lichens, greater moisture, and the lee of prevailing winds. The present writer took systematic notes in permanent sample plots on the relative intensity of infestation on different sides of the tree and found no consistent trend; but observations showed that the sheltered side, as determined by local conditions, is always the most heavily attacked.

Stand Factors

Soil. The relation of quality, tilth, and depth of soil to ability of the tree to withstand attack has not been studied. Hartig (1880) believed that the injury caused by the insect is probably greater on poor soils, but this is more a question of resistance of the tree to attack than of intensity of attack. In this connection Boodle and Dallimore (1911) quoted a letter in which Brotherston stated that the nature of the soil has no influence on resistance of the tree to attack, those growing in pure sand being affected as rapidly as those in a clayey loam. Reh (1927) claimed that injury is greater near large cities, such as Kiel and Hamburg, where leaf litter is not permitted to accumulate and where the visiting public trample the ground to a firm state. Webster (1908) stated that infestation occurs independently of whether the soil be chalky, sandy, loamy, or shaley. The writer has made no special observations on the relation of soil to infestation by *Cryptococcus fagi*.

Density of stand. The number of trees per acre and the closeness of the crown canopy seem to influence infestation only as they affect retention of moisture and protection against driving rains, hot sun, and strong winds.

That there seems to be a relation was stated by Balfour (1863), Kaltenbach (1872), and Piller and Ricker (Hartig 1880). The present writer is convinced that this is an important factor in the forest.

Position of tree in stand. For the same reasons the trees in the interior of a stand are generally more heavily attacked than those at the margins. This was recognized by Ricker (Hartig 1880), Eckstein (1920), Reichling (1920), and Bertelsmann (1913 a) who attributed it to more abundant lichens and softer bark due to greater retention of moisture.

Position of stand. The physiographic position of a stand, *per se*, seems to have little or no effect on infestation. M'Intosh (1849) found attack as uniform on high as on low ground. But where differences in the position of the stand cause changes in moisture and shelter, they influence infestation. Thus Kaltenbach (1872) found infestations to be greater on the shaded side of hillsides. Ricker (Hartig 1880) observed that when stands are sheltered infestation is heavy, independently of aspect and of whether in upland or hollow. Bertelsmann (1913 a) found infestation heaviest in stands having a southwest exposure. The British Forestry Commission (1926) agreed with earlier views that the stands sheltered from prevailing winds are most heavily attacked.

Climate. Climatic limitations are undoubtedly important in restricting the range of the woolly beech scale to parts of a large area such as Europe, where the insect has been present long enough to allow wide distribution. The possibility that changes in weather over short or long periods may affect the activities of an insect such as this one is not to be ignored. Boodle and Dallimore (1911) made the following comment:

"There can also be little doubt that climatic conditions, varying from time to time, favor its increase or decrease . . . . Thus, drought and mild winters would appear to provide the exact conditions under which it is possible for the Coccus to thrive, whilst enfeebled health of the trees (due primarily to drought and in some cases accelerated by old age) makes them peculiarly susceptible to insect attacks."

Rhumbler (1915), on the other hand, held that meteorological influences are of no importance because they act over large areas, and local differences in infestation are evidence that factors other than this are more important. The present writer agrees that local conditions exert a strong influence on infestation; but this is scarcely evidence that climatic conditions and changes in weather from year to year are without effect on the activity, fecundity, and dissemination of the insect. But this is pure conjecture. The writer has studied the disease for too short a period to allow of significant conclusions.

B. THE FUNGUS

HISTORY AND RANGE

It cannot be stated precisely when the fungus which is causing the death of *Cryptococcus*-infested beech in the northeast first appeared, nor when it first

attracted attention, nor when it was first recognized as a species of *Nectria*. Because fatality became sufficiently conspicuous early in the last decade to attract local attention, it is probable that the fungus had been present for some years previously. So far as is known, the first report of a definite fungus associated with mortality, and its recognition as a *Nectria*, following observations in 1929 by Faull (1930) in Nova Scotia, was by the present writer (Ehrlich 1931), who collected it during 1930 in various regions throughout Nova Scotia and in Westmoreland and Albert Counties, New Brunswick. Its progress during the last three years has been followed, and by the close of 1932 the fungus had been observed in several hundred areas in Nova Scotia, including Cape Breton Island; in parts of Westmoreland and the maritime portions of Albert County, and sparsely in Queens County, New Brunswick; commonly in Washington County, and in one stand in Waldo County, Maine.* The amount and age of the fungus in these areas indicate that it is spreading rapidly in the wake of *Cryptococcus fagi*. Many of the scale-infested stands in eastern Massachusetts have been examined repeatedly and in no instance has this fungus been found. Its general range in Nova Scotia, New Brunswick, and Maine is shown in Fig. 1.

Identity

The fungus is a member of the Ascomycetous order Hypocreales, family Nectriaceae, genus *Nectria* Fries (*Creonectria* Seaver). It has been impossible as yet to determine either the precise identity of the species of *Nectria* concerned or whether it is a new species. The morphologic differences in ascospore characters and fruiting bodies among many forms in this genus are so slight that inconsistency and uncertainty have characterized the determination of species, and that cultural characters and the conidial stage have been accorded increasing importance in the recent studies of Wollenweber (1913, 1917, 1924, 1926, 1928 a, 1928 b, 1931) and Richter (1928). The fungus involved in the present investigation has been determined to be a close relative of the common, weakly parasitic, small-ascospored species, *N. coccinea* (Pers.) Fries, as delimited by Wollenweber (1928 a) and Richter (1928). Wollenweber, himself, examined a dry specimen of this fungus in 1929 and identified it as this species. As a result of extensive culturing and examination, the opinion is ventured by the present writer that this fungus differs sufficiently from *N. coccinea* to raise the question of its possible varietal status. Without further comparative studies, however, neither a clear differentiation nor a new name is proposed. In the more general characters of pathogenicity, perithecial color, perithecial size and shape, and growth in culture, it agrees well with the basic species, *N. coccinea* (Pers.) Fries, and its conidial stage, *Cylindrocarpon candidum* (Link) Wr. In addition, the ascus has the characteristic elongate flat top of this species; the ascospore length agrees satisfactorily (the mean of 590 measurements on 16 specimens being 11.9μ, in comparison with Wollenweber's mean of 11.4μ and Richter's, of 11.6μ); and the slightly curved outline of the *Cylindrocarpon* conidia and their width of

*The infected areas in Maine were discovered by Mr. H. B. Peirson, the State Entomologist.

5.0μ–6.0μ are in agreement with similar characters of *N. coccinea*. With *N. coccinea* (Pers.) Fr. var. *longiconia* Wr., the beech *Nectria* agrees in the punctate endospore membranes of the ascospore and in the occasional presence of chlamydospores. The mean lengths of the macroconidia are exceptionally great, even greater than those reported for this variety. Some comparative measurements are given in Table XI. The color of the mycelium

TABLE XI

COMPARISONS OF MACROCONIDIAL MEASUREMENTS OF *N. coccinea* VAR. *longiconia* AND THE *Nectria* ON *Fagus* IN EASTERN AMERICA

Number of septa	*N. coccinea* var. *longiconia*		*Nectria* on *Fagus* in America
	Richter	Wollenweber	Ehrlich
3	38×5.3 (29–50×4–6)	38×4.2 (20–54×3.5–5)	—
4	55×5.8 (40–77×4.7–7)	—	62×5.0 (48–82×4.2–6.0)
5	68×5.9 (50–82×5–7)	64×5.8 (51–75×5–6)	80×5.3 (50–115×4.6–6.0)
6	81×6.5 (73–93×6–7.5)	—	91×5.7 (59–120×5.1–6.2)
7	92×6.2 (83–106×5.7–6.5)	84×5.8 (75–100×5–6.5)	100×6.0 (66–125×5.3–6.6)
8	100×6 (97–111×5.5–6.5)	—	—
9	—	101×6.0 (82–110×5–7)	—

of the beech *Nectria* in culture is distinctive, being white for the first few days, and then turning to various shades of red brown; this character distinguishes it not only from all other species grown by the present writer, but also from *N. coccinea* and its varieties as described by Wollenweber (1928); in this it resembles his *N. punicea* (Schm.) Fr. The beech *Nectria* hardly agrees with Seavér's (1910) description (ascospores 12–16μ in length) of *Creonectria coccinea* (Pers.) Seaver, or indeed with that of any other of his North American Nectriae.

Until more is learned concerning other European and American forms (a subject under investigation by the writer), it cannot be known whether this species is a native one which has been favored recently in the northeast by the increasing activity of *Cryptococcus fagi*, or an introduced species uncommon in Europe which has found scale-infested bark of *Fagus grandifolia* under climatic conditions in northeastern America a more favorable substratum than that of *F. sylvatica* in western Europe.

DESCRIPTION

The perithecia vary in shape from globose to ovoid and taper somewhat apically into an ostiolar papilla whose cells exhibit weakly radial arrangement. They are borne in clusters on discoid, subphellar, erumpent stromata. When fresh, their color is a bright, light red; with age, they darken to a drab, dark, fuscous shade. Their size ranges mostly between 200 and 300μ in width (mean 286μ, extremes 115–397μ), and between 250 and 400μ in length (mean 346μ, extremes 167–496μ).

The asci (Fig. 11 A) are eight-spored, the spores being uniseriately arranged until maturity, when the terminal two or three are crowded laterally into the

apex. The ascus is terminated by a sterile truncate apical portion which is occupied by the spores shortly before they are discharged.

The ascospores (Fig. 11 B, C) are oval to elliptical in outline, two-celled, slightly constricted at the septum; in germination they swell and increase in width before terminal or lateral germ tubes evaginate. The endospore membranes are more or less distinctly punctate or minutely echinulate. The mean length of 590 ascospores was found to be 11.9μ, with a maximal range of 7.3–16.1μ and the means of individual samples ranging between 10.5 and 12.7μ. The widths of 415 ascospores had a mean of 5.7μ with extremes of 4.1 and 7.5μ, the sample means between 4.8 and 6.2μ; these figures may be somewhat high owing to swelling attendant on incipient germination in certain samples.

Microconidia (Fig. 11 D) are formed soon after germination of the ascospores. They are borne aerially on the mycelium on the tips of short branches and are typically unicellular; occasionally they become one or more septate, especially before germination, and grade into the shorter macroconidia. Their germination is sometimes attended by fusion of the germ hyphae (Fig. 11 E).

The macroconidia (form genus *Cylindrocarpon* Wr.) are produced in nature on short conidiophores arising from a subphellar erumpent sporodochium; they are hyaline and appear white in mass. In agar cultures they arise at first on the aerial mycelium and later on small sporodochia from which they well forth as white to golden

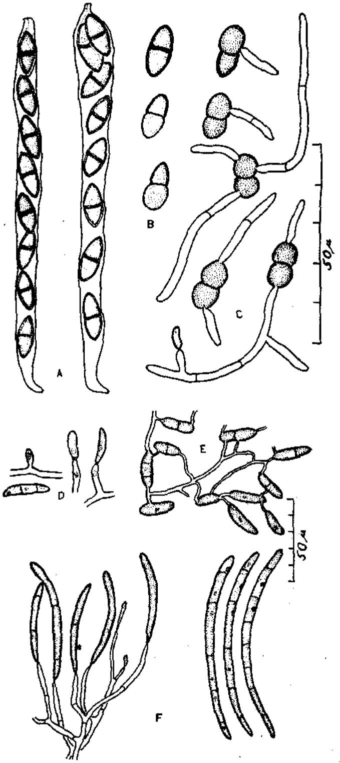

FIG. 11. *A. Asci of Nectria. B. Ascospores. C. Germinating ascospores. D. Microconidia. E. Occasional fusion of microconidial germ tubes. F. Macroconidia.*

yellow pionnotes which rise in columns to a height at times of a centimetre and dry as strands which tend to split apart. The macroconidia are elongate, slightly curved, usually three- to nine-septate (mostly five- or six-), rounded at the distal extremities, and truncate or occasionally slightly swollen at the proximal end (Fig. 11 F). Some measurements of macroconidia are summarized in Table XII. The majority range in length between 60 and 90μ; in width, between 5 and 6μ. Standard errors were computed for most of the sample means and found in all cases to be approximately 1% of the means.

TABLE XII

MEASUREMENTS OF *Cylindrocarpon* CONIDIA

Number of septa	Lengths					Widths			
	Number measured	Number of samples	Mean length, μ.	Range of sample means, μ.	Maximal range, μ.	Number measured	Number of samples	Mean length, μ.	Maximal range, μ.
4	100	2	62	—	48– 82	20	1	5.0	4.2–6.0
5	549	7	80	67– 93	50–115	40	2	5.3	4.6–6.0
6	343	5	91	84–101	59–120	20	1	5.7	5.1–6.2
7	139	3	100	90–108	66–125	20	1	6.0	5.3–6.6

ARTIFICIAL CULTURE

This species has been isolated repeatedly from ascospores and macroconidia produced on *Fagus* bark, and from infected bark and wood; monosporial and polysporial isolations have been made; subcultures from microconidia, macroconidia, ascospores, and mycelium produced in culture have been maintained. The media employed included autoclaved twig and branch sections of *Fagus grandifolia* and *F. sylvatica*, potato plugs, Cayley's glycerine potato plugs, potato agar, potato dextrose agar, rice agar, rice dextrose agar, cornmeal agar, oat agar, prune agar, and Czapek's agar. Although growth occurred on each of these media, the most vigorous and fecund aerial and surface mycelium developed on *Fagus* twigs and on the potato and prune agars. The simplest and most satisfactory medium was potato dextrose agar; this has been used for maintaining stock cultures. Transfers held at 33° C. made practically no growth; those at 27° C. began growing more rapidly than others kept in the laboratory, but were overtaken after a week by the latter.

In artificial culture on potato agar, a white aerial and surface mycelium develops, producing microconidia aerially in the first week. As the mycelium advances, the older portions become yellowish and then reddish brown, and the same colors infuse the agar. In about one month most cultures produce small reddish stromata at the surface of the substratum. Some of these become covered with white aerial hyphae; others develop into sporodochia on which macroconidia are produced, usually in viscous white, cream-colored, or golden streams which rise from the surface as typical pionnotes (Plate IX, Fig. A) and ultimately dry *in situ*, sometimes bent (Plate IX, Fig. B), some-

times split into longitudinal shreds. Perithecia are not produced consistently, but frequently mature at the bases of the pionnotes, in clusters on other stromata, or singly on the surface mycelium; ascospores are usually discharged in drying slant cultures or Petri plates as plugs or tortuous tendrils (Plate IX, Fig. C).

Since field studies during the winter months were not made, the possible influence of low temperatures on ascospore discharge and germination, and on macroconidial germination was examined in the laboratory. In an ascospore test, temperatures between 0° C. and 14° C. were maintained in refrigerators which fluctuated less than 1° C.; the 20° C. level was held in a water bath; and the 24° C. level was approximated in an insulated chamber in the laboratory. The procedure consisted in holding a piece of bark bearing ripe perithecia at the appropriate temperature for an hour before the start, then placing it on moist filter paper in the lower half of a Petri plate and covering with a Van Tieghem ring and cover slip; examination was made by removing the ring and focusing on the underside of the cover slip with a microscope. The data on this test are neither sufficiently numerous nor the conditions adequately controlled to warrant quantitative consideration; they show that ascospores are discharged at temperatures decreasing almost to the freezing point of water and that between 12° and 24° C. the rate of germination tends to increase with temperature.

In tests with freshly discharged ascospores in hanging drops, delayed and sluggish germination took place in a small proportion of the spores at 8° C. Between 12° and 24° C. the mean rate of growth of the germ tubes tended to vary with temperature.

Macroconidia will germinate at temperatures as low as 3° C. The results of a test with macroconidia from fresh pionnotes in culture, germinating at low temperatures in hanging drops, show that both duration of the period before germ-tube emergence and the percentage of spores germinating vary with increases in temperature, the former decreasing, the latter increasing.

Since the violence of ascospore discharge is a factor determining the importance of air currents as an agent of dissemination, several tests were made of the vertical height to which ascospores are raised when discharged from perithecia on small cubes of bark placed in Van Tieghem cell moist chambers. The distances were measured by building up the rings to graded 0.5 cm. heights and examining the cover slips for spores. In such tests the spores often reached a cover slip raised 2.5 cm. above the perithecia, but never higher ones.

Frequent observations indicated that spores at the surface of a drop germinate more rapidly than submerged individuals, a situation probably related to aeration. The relation of the distance of the spore from the atmosphere, to germination, was measured by placing a cover slip over a drop suspension of freshly discharged ascospores and making observations, at points on a straight line across the cover slip, on percentage of germination

and length of germ tubes after a given interval of time. Both measurements decrease with increased distance from the exposed edge of the cover slip.

The possible influence of hydrogen ion concentration was examined by placing a drop of uniform macroconidial suspension in the centre of each of a series of eight Petri plates ranging in pH from 2.2 to 9.6, and holding the plates at 12° C. In this test the fungus was able to develop between pH limits of 4.5 and 9.6, maximum growth taking place at 8.0.

SPORE LIBERATION AND DISSEMINATION

Since it had been learned that ascospores are forcibly discharged from mature perithecia whenever they become moistened, it was decided to examine the occurrence of discharge in the field and its possible relation to weather conditions. The procedure consisted in placing traps over clusters of ripe perithecia on infected bark and making periodic microscopic examinations, as well as observations on general weather conditions and moistness of the bark. The trap was an ordinary 2 by 1-in. microscope slide held in place on a wood lath by a strip of aluminium tagging tape bent at both ends; the lath was secured to the trunk by a single large nail which permitted swinging into position during a test, and away from the discharge area for changing slides. With copious discharge, slides frequently became covered so densely with ascospores as to appear splashed with whitewash. The results of one such series of tests with 12 traps during the summer of 1931 in Kings County, Nova Scotia, are presented in Table XIII. The last days of July were dry, with the exception of showers on July 26, which failed to wet the bark. In order to be certain that the apparently mature perithecia were actually capable of spore discharge, four of the test areas were slightly moistened artificially, and two of them gave appreciable ejections. The first rain (on August 4) of sufficient magnitude to wet the trunks of the beeches under examination resulted in copious discharges which dwindled progressively on the following two days, although they continued lightly on certain areas even after the surface of the bark appeared dry. A lighter rain on August 10 resulted in renewal of discharge on all of the test areas which became wet, and continued on the following day in spite of surface drying.

Weather-vane spore traps were set in conjunction with the foregoing tests to yield information on possible air dissemination of the spores. These traps were modified from similar ones used by the Canadian Department of Agriculture; they consisted of a galvanized iron box open at both ends, with a sliding bottom, and containing centrally on the two sides two slotted holders for a microscope slide; one end was equipped with a large vertical sheet of metal for a weather vane, and the other accurately counterbalanced with lead. The trap was suspended by a hook soldered into the top, and a chain made by twisting baling wire into large rings. It was suspended from a branch and guyed to prevent swaying. This method of suspension was found to be far more sensitive in orientational response to the lightest air currents than the usual more complicated, heavy, and expensive upright ball-bearing equipment. The microscope slide in such a trap always faces

TABLE XIII

ASCOSPORE DISCHARGE RECORDS; KINGS CO., N.S.; JULY-AUGUST, 1931

Month	July					August						
Date	25	26	27	28	31	4	4	5	5	6	10	11
Hour	10 a.m.	11 a.m.	6 p.m.	3 p.m.	11 a.m.	11 a.m.	5 p.m.	11 a.m.	7 p.m.	7 a.m.	11 a.m.	11 a.m.
Weather during preceding interim	Sunny, windy, muggy	Showers, muggy	Sunny, windy, hot	Sunny, windy, hot (*a*)	Cloudy, windy, cool	Sunny, cool; 3-4p.m., rainy, muggy	Sunny, breeze, warm	Sunny, breeze, warm	Sunny, breeze, hot	Sunny, breeze, cool	Sunny, rain, cool	Sunny, cloudy
Condition of bark	Dry	Dry	Dry	(*a*)	Dry	All wet	Wet under traps	Moist under some	Dry	Dry	Moist in spots	Dry
Bark traps												
126E–A	0	0	0	*a* 0	0	3	2	0	0	0	*m* 0	0
126E–B	—	0	0	0	0	3	2	*m* 2	1	0	0	0
127E–A	0	0	0	*a* 3	0	3	2	*m* 2	2	1	0	0
127E–B	—	0	0	0	0	3	2	0	0	0	*m* 1	0
128E	0	0	0	0	0	3	3	*m* 3	2	0	*m* 3	3
129E	0	0	0	0	0	2	2	2	2	0	*m* 2	2
130E–A	0	0	0	*a* 0	0	2	2	0	0	0	0	0
130E–B	—	0	0	0	0	2	2	*m* 1	0	0	*m* 1	2
131E–A	0	0	0	*a* 2	0	3	2	2	1	0	0	0
131E–B	—	0	0	0	0	1	1	1	0	0	*m* 1	2
132E	0	0	0	0	0	3	1	*m* 0	0	0	*m* 2	2
133E	0	0	0	0	0	3	2	0	0	0	*m* 3	3

Key to abbreviations: *a, artificially moistened; m, moist; —, no observation; 0, no discharge; 1, light discharge; 2, medium discharge; 3, copious discharge.*

the wind and is protected against being washed by rain and against falling debris. Such traps were hung at various elevations and positions in the stands being tested for ascospore discharge in the hope that they might yield collatable data on air dissemination. No ascospores were caught in the Kings County tests. Subsequent success elsewhere justifies the opinion that the relatively few beeches with mature perithecia in the long-infected and largely decimated stands in Kings County supplied insufficient spores for measurement in so minute a sampling of the atmosphere.

These tests were repeated during August, 1931, in a more recently infected beech stand in Inverness County, Nova Scotia. The results are presented in Table XIV. Here, as in the foregoing tests, rains of sufficient magnitude to wet the bark of the trunks resulted in spore discharge, which continued in some cases for three or four days after the bark surface had become dry. Macroconidia from fresh pustules were present on these trees and were caught in smaller numbers and for shorter periods following the rains. It is not known whether they are abstricted explosively or merely dislodged and caught by air currents; the simple manner of formation on the tips of conidiophores suggests the latter explanation.

Negligibly few spores were caught in the weather-vane traps following the first rain. The catch during and after the second rain on August 26 included ascospores and macroconidia in sufficient numbers to demonstrate conclusively that both spore forms are carried by air currents. It is very probable that when rainy weather lasts for two or more days, enabling discharge, dissemination, and germination in suitable inoculation courts, the dissemination of spores by air currents provides the most important method of spreading the fungus from tree to tree and possibly from stand to stand.

It is not unlikely that animals such as insects, arachnids, birds, and rodents occasionally act as unwitting carriers of the fungus. There is no direct evidence for or against this possibility, but the varied and active arthropod fauna on forest beech strongly suggests its correctness. Tests to determine whether crawlers of *Cryptococcus fagi* carry spores were attempted but yielded no conclusive data. That the crawlers are not specific vectors appears probable to the present writer. Only on heavily infested trees which have become lightly infected by the *Nectria* and support fresh sporodochia and perithecia in limited areas are there still healthy portions capable of nourishing *Cryptococcus* colonies; larvae hatched on such trees undoubtedly carry spores on their bodies and may thus transport inoculum to fresh bark on the same or other trees. That this is an important means of local dissemination appears unlikely.

Long range distribution of the fungus may possibly be effected by wind, birds, or man. Nothing is known of it.

TABLE XIV

ASCOSPORE AND MACROCONIDIUM DISCHARGE AND CATCH RECORDS; INVERNESS CO., N.S.; AUGUST, 1931

Month	August								
Date	20–21	21	21	22	22	24	25	26	27
Hour	5 p.m.-9 a.m.	2 p.m.	5 p.m.	10 a.m.	5 p.m.	10 a.m.	9 a.m.	10 a.m.	2 p.m.
Weather during preceding interim	Rain	Showers, sunny, windy	Sunny, windy	Sunny, breeze	Sunny, breeze	Sunny, breeze	Sunny, breeze, cloudy	Rain	Sunny, breeze
Condition of bark	All wet	Moist in spots	Moist in spots	All dry	All dry	All dry	All dry	All wet	Drying
Bark traps									
134E–A	3	*m* 3	*m* 1	0	0	0	0	3	2
134E–B	3	3	1	0	0	0	0	3	2
134E–C	2 *C**	*m* 3	*m* 2	2	1	1	0	3 *C*	2
135E	2 *C*	1	0	0	0	0	0	2 *C*	1
136E–A	3 *C*	*m* 3 *C*	*m* 3	2	2	2	0	3 *C*	3
136E–B	3 *C*	3 *C*	1	0	0	0	0	3 *C*	3
136E–C	3 *C*	3 *C*	0	0	0	0	0	3 *C*	2
136E–D	3 *C*	3 *C*	*m* 2	1	0	0	0	3 *C*	2
136E–E	2 *C*	*m* 2 *C*	*m* 1 *C*	1	1	0	0	2 *C*	2
137E–A	3 *C*	*m* 0 *C*	0	2	1	1	1	3 *C*	3
137E–B	3 *C*	*m* 2	*m* 2	2	0	0	0	3 *C*	2
138E	3 *C*	1 *C*	0	0	0	0	0	2 *C*	2
***Aerial weather-vane traps*†**									
1	0	—	0	—	—	—	—	11	—
2	0	—	2	—	—	—	—	5	1
3	0	—	1	—	—	—	—	34, 11*C*	3
4	0	—	0	—	—	—	—	77, 4*C*	0
5	0 *C*	—	0	—	—	—	—	54	3
6	0 *C*	—	1	—	—	—	—	8, 5*C*	1

**C* : *macroconidia* (*Cylindrocarpon*). †*Recording actual numbers of spores caught.*

C. PATHOGENICITY

OUTLINE OF THE PROOF OF PATHOGENICITY

The proof of the role played by each of the two pathogens in the beech bark disease rests on extensive field observations and on experiments in the field and greenhouse. The evidence supports the etiologic theory that the woolly beech scale, *Cryptococcus fagi* (Baer.), alone is not fatally pathogenic but is the initial agent, predisposing the tree to infection by the *Nectria*, which—once established—grows parasitically and causes death by destruction of the protective, storage, and vascular systems of the trunk and larger branches. The nature of the evidence adduced in support of this theory is summarized in the following outline.

A. *Cryptococcus fagi* is the initial essential agent under field conditions, but alone does not cause death.

1. *Nectria* infection occurs in nature only on *Fagus* bark contemporarily or previously infected by *Cryptococcus;* mortality typically follows *Nectria* infection.
2. Where *Cryptococcus* is not followed by *Nectria*, death rarely occurs.
3. Field and greenhouse inoculations of clean intact bark failed to produce infection.
4. Preliminary field inoculations of *Cryptococcus*-infested bark with *Nectria* spores resulted in infection.
5. Areas of bark on trunks of uninfested trees, protected against subsequent infestation, remained uninfected, while exposed areas on the same trees became infected.
6. Areas of bark on trunks of artificially disinfested trees, protected against subsequent infestation, became infected, but less abundantly than exposed areas on the same trees.

B. A species of *Nectria (coccinea* group) infects bark infested by *Cryptococcus fagi*, is able to grow parasitically, and is immediately responsible for tissue necrosis and mortality.

1. Signs and symptoms of infection by this species of *Nectria* develop in nature on a preponderant majority of the *Cryptococcus*-infested trees in the regions known to harbor this fungus.
2. The fungus has been repeatedly isolated from naturally inoculated bark and wood which had or still harbored the insect, and has been grown in pure culture to fruiting stages.
3. Artificial field and greenhouse inoculations on intact bark resulted in no infection; but similar inoculations on mechanically wounded and on *Cryptococcus*-infested-bark produced infection, and in some cases fruiting bodies. The fungus was reisolated from infected tissues and fruiting bodies, and grown in pure culture.

Role of Cryptococcus fagi

Field observations

Cryptococcus alone. Many of the early accounts of *Cryptococcus fagi* in Europe report trees infested for periods of one and two decades without suffering apparent injury. In more recent times the conclusion was reached in England by Boodle and Dallimore (1911) and in Germany by Bertelsmann (1913 a), Rhumbler (1914, 1915, 1922, 1931), and others that the insect alone produces little or no damage but that fungi, which gain entrance to the living tissues as a result of insect attack, are the immediate pathogens. No experimental evidence in support of this conclusion has been reported. The same opinion is fully borne out by the situation observed in America by the present writer. Here a particular species of *Nectria* has become so abundant and widespread in Nova Scotia and New Brunswick that it fatally infects trees attacked by *Cryptococcus* with such speed and severity as to have resulted in a veritable epiphytotic. In eastern Massachusetts, on the contrary, the fungus has not been discovered and many trees which are known to have been infested by the insect for at least four years show no serious effects.

Nectria following Cryptococcus. Many fungi have been reported in Europe as causing injury to *Cryptococcus*-infested beech; but among them, beginning with Hartig (1880), species of *Nectria* (usually referred to *N. ditissima* Tul.) have been accorded the greatest importance. It is unlikely that the species which has become widespread in the Maritime Provinces is the same as those found in Europe (as explained in Section VII B, above). This species has been found by the present writer only on bark of *Fagus grandifolia* contemporarily or previously infested by *Cryptococcus fagi*, and only in Nova Scotia, New Brunswick, and Maine. The fungus is associated in the great preponderance of cases with death of the infested trees. This fact is not proof of the contention that *Cryptococcus* is an essential agent in the Maritime outbreaks of the beech disease, but constitutes supporting evidence of experimental proofs submitted below.

Inoculations on Clean Intact Bark

The first question to be decided experimentally was whether or not this *Nectria* is capable of penetrating the corky layer of the bark and producing infection. The possibility was tested with forest beeches at Fredericton, New Brunswick, beyond the range of *Cryptococcus fagi*, and on a great many occasions with potted sapling beeches (both *Fagus grandifolia* and *F. sylvatica*) in the greenhouses at Harvard University.

Field inoculations were made with ascospore suspensions, with pieces of infected bark secured so that the ostioles of mature perithecia were in contact with the inoculation courts, and with macroconidial masses from natural pustules and from pionnotes in culture. The inoculation courts were clear areas of bark which had never been attacked by *Cryptococcus fagi;* they were lichen-free, lichen-covered, or lichen-freed. The inoculum was applied

to marked courts on the previously moistened bark, and covered with layers of wet absorbent cotton and wrapped in factory cotton or oilcloth. They were examined after two months or in the following year. In no case had infection resulted.

Greenhouse inoculations were made with ascospores and macroconidia on lichen-free bark of potted saplings. In the early tests the courts were wrapped with wet absorbent cotton and raffia; in the later ones, the courts were inclosed in celluloid cylinders sealed at top and bottom with wet cotton or sphagnum (Plate IX, Figs. F, G) or the saplings placed in a tent within which the atmosphere was kept saturated by a continuous flow of water over the canvas (Plate IX, Fig. D). Not a single infection developed.

Inoculations on Cryptococcus-infested Bark

If *Cryptococcus*-infested bark known to be uninfected by the *Nectria* could be successfully inoculated, this would be proof that the insect enables the fungus to infect bark otherwise resistant. Such inoculations should be made on trees in a region infested by the insect but free of the fungus. Eastern Massachusetts is an ideal region for this purpose; but the writer refrained from carrying out such an experiment out of doors because of the possibility that the dangerous species of *Nectria* might inadvertently be liberated and become established locally. Field inoculations were made in the Maritime Provinces and greenhouse inoculations at the Arnold Arboretum.

Field inoculations were made in Digby and Inverness Counties, Nova Scotia, and in Albert County, New Brunswick, on recently infested trees which at the time of inoculation showed neither symptoms nor signs of *Nectria* infection. The insects were not disturbed in certain tests; in others they were removed by scrubbing with a contact insecticide followed by an aqueous wash. Inoculation courts were protected against reinfestation and natural fungal inoculation by wrapping in tarred factory cotton (Plate IX, Fig. H) or tar-sealed oilcloth. The great majority of these artificial inoculations were followed by definite lesions and fruiting bodies of the *Nectria*. But the conclusiveness of these results is not entirely satisfactory because in many cases the bandaged control areas, consisting of insect-infested bark not artificially inoculated, also became infected, indicating that natural inoculation had preceded the artificial tests. Unquestionably this experiment should be repeated in an area where there is as yet no possibility of natural *Nectria* infection. Nevertheless, successful inoculations with uninfected controls, secured in certain of the tests, are reliable evidence that the fungus is able to infect insect-infested areas of bark, since even a single clear cut positive result is significant in itself, and the contamination of other tests is not evidence against the positive result.

Greenhouse inoculations. The difficulty of finding a satisfactory location for field inoculations in the Maritime Provinces, and reluctance to perform them in eastern Massachusetts increased the desirability of greenhouse experiments where the danger of liberating the fungus could be held at a

minimum. In 1932, pieces of heavily infested bark harboring hatching eggs were removed and tied to potted saplings in the greenhouse. An abundant transfer of crawlers took place and many settled on the saplings and secreted wool, the number being greatest on saplings kept in the moist chamber tent. As yet the insect has not become sufficiently established to warrant performing inoculations.

Protection Experiments on Uninfested Bark

If areas of bark on as yet uninfested or newly and lightly infested trees in regions recently attacked by *Cryptococcus fagi* and the *Nectria* could be protected from subsequent infestation, but not from *Nectria* inoculation, the absence of *Nectria* infection on these areas, at the same time that adjacent unprotected areas on the same trees became infested and infected, would constitute additional evidence in support of the view that the insect is an essential initial factor in *Nectria* infection. This possibility was tested by selecting two uninfested trees and two very lightly infested ones on which the insects were removed from the area to be protected; an area on the trunk three feet vertically at convenient height was wrapped in phosphor-bronze wire cloth (120 mesh per inch) in such a way that it came into contact with the bark only at the top and bottom ends, which were coated with Tanglefoot (Plate IX, Fig. I). This material was renewed occasionally. A few nymphs were able to crawl through infrequent larger apertures, but for the most part protection was excellent. Examination after 15 months consisted in counting the numbers of lesions visible on the surface of an area two feet high under the screen, and an area one foot high immediately above the screen, shaving the bark below the phelloderm and counting (Plate IX, Fig. J), and finally removing the bark and counting the lesions visible on the surface of the wood. The pertinent data on this experiment are presented in Table XV. It will be seen from the table that although a few small

TABLE XV

EFFECT ON *Nectria* INFECTION OF PROTECTING BARK AGAINST *Cryptococcus* INFESTATION

Tree no.	Protected May 12, 1931.	Examined August 25, 1932				
		Protected area		Adjacent unprotected area*		
					Nectria infection	
	Cryptococcus infestation	*Cryptococcus* infestation	*Nectria* infection	*Cryptococcus* infestation	Lesions in bark	Lesions to wood
213 E	None	Very light	None	Medium	5	3
214 E	None	Very light	None	Medium	0	0
237 E	Very light (removed)	Very light	None	Medium	>200	90
238 E	Very light (removed)	Very light	None	Medium	>200	56

*Since the area examined was only half as great as that under the screen, the number of lesions has been doubled.

colonies of the insect developed in the protected areas, not a single *Nectria* infection resulted, while in the adjacent unprotected areas on the same trees the insect increased in numbers and fungal lesions appeared in varying quantities. This is taken to be evidence supporting the view that the continued feeding of *Cryptococcus fagi* enables otherwise impossible *Nectria* infection.

Protection Experiments on Disinfested Bark

A similar experiment was made on trees infested with *Cryptococcus* but exhibiting neither symptoms nor signs of *Nectria* infection. Five trees were selected, ranging in amount of infestation from light to heavy. The insect was removed from an area of trunk surface three feet vertically, and the area protected with a phosphor-bronze screen. When the trees were examined after 15 months (Table XVI), reinfestation of the supposedly protected areas was observed to have taken place to a small extent; but, in spite of this, the results, although variable quantitatively, showed a consistently greater infection of the adjacent unprotected areas than of those protected. This experiment, also, is interpreted as evidence supporting the contention that under forest conditions the ability of the *Nectria* to infect is dependent on feeding by *Cryptococcus fagi;* it shows, further, that previous feeding by the insect—when of sufficient intensity and duration—makes possible subsequent fungal infection.

TABLE XVI

EFFECT ON *Nectria* INFECTION OF PROTECTING PREVIOUSLY INFESTED BARK AGAINST *Cryptococcus* INFESTATION

Tree no.	Disinfested and protected May 13, 1931. *Cryptococcus* infestation	Examined August 22, 23, 1932 — Protected area: *Cryptococcus* infestation	Protected area: *Nectria* infection, Lesions in bark	Protected area: *Nectria* infection, Lesions to wood	Adjacent unprotected area*: *Cryptococcus* infestation	Adjacent unprotected area*: *Nectria* infection, Lesions in bark	Adjacent unprotected area*: *Nectria* infection, Lesions to wood
261 E	Light	Light	78	6	Medium	>200	20
262 E	Light	Light	6	6	Medium	>200	104
304 E	Medium	Light	24	0	Medium	140	0
284 E	Heavy	Light	9	0	Heavy	>200	26
285 E	Heavy	Medium	88**	21	Extra heavy†	much >200††	>200

**Since the unprotected area examined was only half as great as that under the screen, the number of lesions has been doubled.*

***Twenty-four surface lesions with Cylindrocarpon sporodochia.*

†*Many colonies already dead because of drying of substratum.*

††*So largely coalesced that 90% of area necrotic; many surface lesions with sporodochia and fresh perithecia.*

ROLE OF NECTRIA

Field Observations

The symptoms of this disease develop only in regions known to harbor the particular species of *Nectria* (*coccinea* group) under discussion, and in

these regions only on trees infected by this fungus. Development of this fungus, observed in nature exclusively on trees attacked by *Cryptococcus fagi*, precedes the appearance of systemic symptoms of mortality (in the crown), continues with the progress of such symptoms, and ceases soon after the production of holonecrosis in the immediate substratum, and death of the tree. Such behavior is characteristic, not of a saprophyte, but rather of a parasite lacking only the ability to effect penetration of normal intact bark. These observations alone are not proof of the pathogenicity of the fungus, but strongly support the experimental evidence.

Isolation from Naturally Infected Tissues

Additional evidence, in agreement with experimental results, is afforded by the constant association of the fungus with necrotic lesions on the bark of *Cryptococcus*-infested beech. Conidial and ascigerous fructifications of the *Nectria* alone develop on the great majority of such lesions; when the fungus is artificially cultivated from the spores of such fructifications, spores are produced in culture. Aseptic tissue plantings from such lesions, and in the Maritime Provinces from the freshly necrotic margins of slime-flux lesions, regularly give rise to typical fruiting cultures of the fungus, similar in every respect to those secured from spore isolations.

Inoculations and Reisolation

Inoculations on intact bark. It has been pointed out above that inoculations with macroconidia and ascospores of the *Nectria* on clean intact *Fagus* bark fail consistently to result in infection. Although this is negative evidence, the fact that other inoculations on ruptured bark were successful lends reliability to the negative results. They are interpreted to demonstrate that the fungus is incapable of infecting sound bark of beech.

Inoculations on mechanically wounded bark. If the theory that the feeding of *Cryptococcus fagi* exerts no specific chemical or biological influence on the resistance of the bark to *Nectria* infection, but merely breaks down the mechanical barrier of the phellem, be true, artificial inoculation of mechanically injured bark should result in infection. Such infection would also be evidence of the ability of the fungus to grow parasitically, once the phellar barrier is broken. Accordingly, healthy bark was subjected to injury ranging from superficial needle punctures of the phellem to scalpel slits penetrating to the cambium. The methods of inoculation were similar to those described above. The inoculations, performed in affected parts of the Maritime Provinces, were protected against subsequent natural inoculation by wrapping with tarred factory cotton or tar-sealed oilcloth. Others were made at Fredericton, New Brunswick, beyond the range of the pathogens. Still others were set up in the greenhouses at Harvard University. These inoculations were followed almost invariably by infection; further, the fungus spread for distances attaining several centimetres periclinally, and often into the wood. The appearance of one trunk, wounded before inoculation by scraping of the periderm, showed, when examined after nine months, that infections had pene-

trated in several patches to the surface of the wood (Plate IX, Fig. E, photographed after bark had been peeled from the wood). Greenhouse inoculations were frequently followed by the production of macroconidial sporodochia and mature perithecia on the infected tissues; in certain cases the fungus maintained itself and advanced slowly for two or three years, and could be induced to fruit again by inclosing the infected area in a celluloid cylinder moist chamber or placing the saplings into the moist chamber tent. These experiments, together with the results of inoculating *Cryptococcus*-infested bark, yield evidence that the role of the insect is mechanical rupturing of the periderm, attendant on death and shrinkage of the probed tissues. They demonstrate, further, that the fungus is able, under experimental conditions, to infect bark whose periderm has been ruptured, to spread parasitically, and to fruit.

Inoculations on Cryptococcus-infested bark. The results of preliminary inoculations on *Cryptococcus*-infested bark, described above, contribute to the evidence that the *Nectria*, although incapable of penetrating intact bark, is able to infect tissues on which the insect has been feeding.

Reisolation of the fungus following artificial inoculation. Following a number of the inoculation experiments, the fungus was reisolated, both from infected tissues and from macroconidia and ascospores produced on the infected tissues. The fungus was grown in pure culture and its mycelium and spores found to be entirely similar to those in original cultures. In a few cases, inoculations with spores from the same reisolation cultures were made; infection resulted and the fungus was reisolated a second time. These results are regarded as final proof that the species of *Nectria* under consideration is able to infect *Fagus* bark whose periderm has been ruptured either artificially or by tearing of the periderm attendant on death and shrinkage of *Cryptococcus*-probed tissues.

SUMMARY

It has been demonstrated that the beech bark disease in northeastern America results from the sequent activity of two pathogens, the woolly beech scale, *Cryptococcus fagi* (Baer.), and a species of *Nectria* (*coccinea* group). The evidence for the role of each of these organisms, outlined at the beginning of this section, consists of (1) constant association of the organisms with the disease, (2) the absence of the disease when only one is present, (3) the consistent isolation of the fungus from infected tissues, and (4) the inability of the fungus to infect tissues not infested by the insect, and its ability to infect those infested. The role of the insect has been shown to be the rupturing of the periderm attendant on death and shrinkage of the living cells of the bark on which the insect feeds. This enables initial infection by the fungus. The role of the fungus is the causing, by parasitic growth, of death and destruction of the storage and vascular systems of the trunk and branches.

D. COURSE OF THE DISEASE

Protection Experiment

The question of determining how long a period of time elapses between first attack by the insect and initial fungal infection is partially elucidated by a field experiment. Eight small experimental plots were selected, ranging in amount of *Cryptococcus* infection from none to heavy, and in duration to at least three years, and showing no indications of *Nectria* infection. Areas extending three feet vertically on the beech in these plots were disinfested by scrubbing (except the originally uninfested trees) and were protected against subsequent infestation and infection by wrapping in tarred factory cotton or oilcloth. These areas and adjacent unprotected areas on the same trees were examined after 15 or 23 months by searching the bark surface for *Cryptococcus*, and for signs and symptoms of infection, then shaving the bark below the periderm and counting lesions, and finally removing the bark and counting lesions on the wood. The trees in these plots have been arranged in five classes based on initial *Cryptococcus* infestation, and the data secured at the conclusion of the experiment summarized in Table XVII. Examining the *Cryptococcus* columns, it will be seen that protection was imperfect with three trees; in all others the protected areas remained free of the insect.

TABLE XVII

Development of *Cryptococcus* and *Nectria* on protected and unprotected areas of trees with varying initial *Cryptococcus* infestation

Initial *Cryptococcus* infestation*	No. of trees	**	At conclusion of experiment						
			Per cent of trees with *Cryptococcus*					Number of *Nectria* lesions per area per tree	
			0	1	2	3	4	In bark	To wood
0	41	P†	100	0	0	0	0	2	1
		U	32	12	44	7	5	17	1
1	27	P	96	0	4	0	0	13	4
		U	11	0	30	59	0	58	19
2	22	P	91	0	9	0	0	29	12
		U	9	0	5	77	9	91	42
3	9	P	100	0	0	0	0	40	11
		U	0	0	0	67	33	121	25
4	15	P	100	0	0	0	0	68	22
		U	0	0	0	47	53	198	102

	0	*1*	*2*	*3*	*4*
**Amount of infestation*	*None*	*Very light*	*Light*	*Medium*	*Heavy*
Duration of infestation	—	*Few months*	*Approx. 1 year*	*Approx. 2 years*	*> 2 years*

**P = *protected;* U = *unprotected.*

†*No signs or symptoms of Nectria infection when protected.*

The infestation on adjacent unprotected areas increased progressively with the amount and duration of initial infestation, except on the most heavily attacked trees; the infestation on certain of the trees in each class, except the uninfested, decreased, however, a situation related to death of infested areas of bark following their infection by the *Nectria*. Turning to the infection figures, it is evident that the number of *Nectria* lesions on protected bark also increased progressively with the amount and duration of initial *Cryptococcus* infestation, being a probably negligible 2 per area per tree on areas uninfested at the start of the experiment, 13 on areas very lightly infested for only a few months, 29 on areas lightly infested for approximately one year, 40 on areas generally infested for approximately two years, and 68 on areas heavily infested for over two years (Fig. 12). The number of these lesions penetrating to the wood is less on most trees. These figures permit no precise determination of the period elapsing under forest conditions between first insect attack and fungal infection. It will be observed, however, that not only are the numbers of lesions small on protected areas infested for less than one year at the outset, but also ratios of lesions on unprotected to protected areas are larger for the areas initially infested for less than a year ($8\frac{1}{2}$ to 1, and $4\frac{1}{2}$ to 1) than for the areas initially infested for a year or longer (approximately 3 to 1). Both of these relations suggest that the fungus infects extensively only where the insect has been present for a year or longer. This interpretation is also borne out by the data in Tables XV and XVI, which show that, with artificially disinfested bark protected against insect attack but not against fungal invasion, subsequent infection took place only when the infestation had continued for approximately one year or longer.

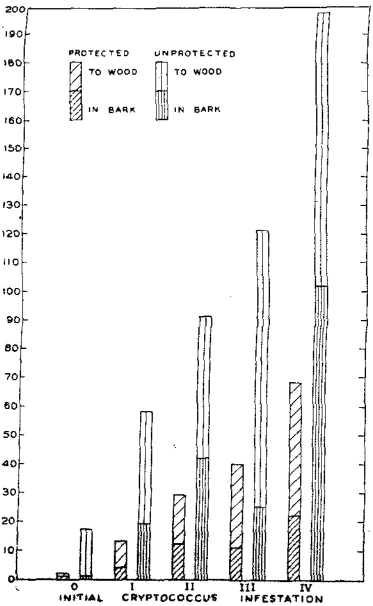

FIG. 12. *Development of Nectria on protected and unprotected areas of trees with varying initial Cryptococcus infestation.*

Permanent Sample Plots

In order to make careful and consistent observations on the development of the pathogens and the course and duration of the disease on trees of different ages in various types of stands, nine permanent sample plots were laid out, in which 1425 beech were tagged and described. Observations were made in most of the plots in each of three consecutive years and the data on each tree recorded on a specially designed, mimeographed form sheet. The plot data recorded include size, type, site quality, density, slope, aspect, drainage, soil, humus, slash, ground cover, underbrush, reproduction, and miscellaneous observations on the stand, its pathological condition, and the presence of *Cryptococcus*, *Nectria*, and other insects and fungi. The data recorded for each tagged tree include crown class, diameter (d.b.h.), various notes on foliage, branches, trunk injury, bark, mosses, lichens, *Cryptococcus fagi*, the predacious Coccinelid: *Chilocorus bivulnerus* Muls., beetle borings, other insects, slime-flux lesions, signs and symptoms of *Nectria* infection, and other fungi.

In order to show something of the information available from this mass of data, a few pertinent facts are presented on Plots 1 and 7. Certain general data appear in Table XVIII. The plots, situated in widely separated parts of Nova Scotia, are of uniform area and site quality. Plot 1 is on a steep slope, and Plot 7 is almost level. Plot 1 is composed of almost pure beech, and Plot 7 is in a typical mixed tolerant-hardwoods type. The crown densities of the two plots are similar. Plot 1 contains a much larger proportion than Plot 7 of suppressed trees. When the sample plots were laid out and first examined in 1930, Plot 1 was selected as representative of an early stage of the disease; although every tree supported the woolly beech scale, only 8.5% showed indications of *Nectria* infection and none had died. Plot 7 represented an advanced stage of the disease; many trees were already dead for more than a year and are not included in the present account; all beech were or had been infested by *Cryptococcus*, 69% were already infected by *Nectria*, and 4.8% had died of the disease in the preceding year. In 1931 (Table XIX), the amount of living insects decreased with death of certain of the affected trees; the percentage of infected trees increased sixfold in Plot 1, and by 10% in Plot 7; 2% of the trees died of the disease in Plot 1, and 11% more in Plot 7. By 1932, the percentage of infected trees had reached 76 in Plot 1 and 92 in Plot 7; mortality due to the disease claimed 3.4% in Plot 1 and 59% in Plot 7.

TABLE XVIII

GENERAL DATA ON PERMANENT SAMPLE PLOTS 1 AND 7

	Plot 1	Plot 7
Location	Inverness County, N.S.	Annapolis County, N.S.
Size	0.5 acre	0.5 acre
Site quality	I	I
Slope	100%	3%
Type	Pure beech	Beech, birch, maple
Crown density	.7	.7
Number of beech	294	147
Number (exclusive of suppressed) of beech	59	82

These data from typical beech stands show several facts regarding the beech bark disease. Those from Plot 1 indicate that, once the trees become generally infested by *Cryptococcus fagi*, *Nectria* infection develops sufficiently to produce external signs and symptoms on the majority within three years and kills some of them in one or two years. The Plot 7 data show that in the later stages of infection the majority of susceptible trees have already been infected, but that, in spite of the slower increases, almost an entire stand becomes infected. In the later stages, the amount of infection on *Nectria*-attacked trees builds up rapidly and leads to large increments in mortality.

TABLE XIX

PERCENTAGE OF BEECH INFESTED, INFECTED, AND KILLED IN PLOTS 1 AND 7

	Plot 1			Plot 7		
	1930	1931	1932	1930	1931	1932
Cryptococcus fagi (living or remains)	100	100	100	100	97	98
Cryptococcus fagi (remains only)	0.0	5.8	7.5	5.4	28	50
Nectria (symptoms or signs)	8.5	5.3	76	69	88	92
Killed by *Nectria*	0.0	2.0	3.4	4.8	16	59
Killed by all causes	0.0	5.8	7.5	5.4	22	64

When the dead beech are grouped according to size and the percentages of trees in each diameter and crown class dead computed (Table XX), the fact emerges that the percentage of beech dying as a result of the disease increases

TABLE XX

PERCENTAGE OF BEECH KILLED BY *Nectria* COMPARED WITH DIAMETER AND CROWN CLASS (PLOTS 1 AND 7, 1932)

Plot		Diameter classes				
		Sapling	1–2 in.	3–6 in.	7–10 in.	11–16 in.
1	Number of beech	102	96	51	28	17
	Per cent dead	1.0	11	39	43	35
7	Number of beech	—	39	66	31	11
	Per cent dead	—	31	51	94	100

Plot		Crown classes			
		Suppressed	Intermediate	Co-dominant	Dominant
1	Number of beech	235	17	18	24
	Per cent dead	11	41	50	38
7	Number of beech	67	28	23	29
	Per cent dead	43	75	83	93

with size of tree. By both measures of size, the largest class in Plot 1 is an exception to this trend, its percentage of beech dead being less than that of the next smaller class. The explanation of this situation may lie in the fact that the larger older trees are less severely infested and infected than somewhat smaller ones, probably because of their rougher, more corky bark. The figures for Plot 7, which has been attacked for a longer period than Plot 1, bear out this explanation both in their consistent upward trend and in their levelling off with higher size classes. These figures are based on relatively few trees, but the analysis of line-plot data (Section VIII, below) on several thousand individuals reveals the same trend.

VIII. Factors Influencing Development of Infection and Mortality

Two schools of thought exist with regard to the evaluation of the agencies which combine to induce the pathic changes which constitute a disease. One school places primary emphasis on the immediate causes of a disease, whether they be biotic or abiotic. The other school admits that the primary causal agent is a factor *sine quo non*, but contends that the complexes of other abiotic and biotic factors which influence the virulence of a disease are the more important, both scientifically and economically, since it is they which often determine the actual course and consequences of a disease. It is a fact that diseases vary in the extent to which immediate and secondary factors influence their course: in some, as with the chestnut bark disease, the mere presence of the causal fungus *(Endothia parasitica)* is sufficient to cause infection and ultimate death, regardless of such variables as age of tree, local environment, and weather; in others, the pathogen is more or less continually present and the disease becomes virulent only under particular conditions of weather (late blight of potato) and age of tree (white trunk rot of poplar). The influence of such factors can be exerted on pathogen (as with late blight), on suscept (as with white rot), or on both.

With the disease discussed here, as with others, the existence of such influences is of technical interest and possibly of practical importance in governing control measures. The influence of such factors on the habits and development of insect and fungus has been discussed in Section VII. Certain of the factors which affect the course of infection and mortality are dealt with in the following pages.

PRELIMINARY OBSERVATIONS

Preliminary observations in the course of two summers' field work led the writer to the conviction that three factors especially influence the development of the fungus and the disease; these are (1) density of stand, (2) the amount of mosses and foliose lichens on the trunk, and (3) size of tree.

It appeared that in stands with a dense crown canopy, where sunlight and warmth penetrate less readily, where drying winds are less effective, and where moisture following rains remains longer, the fungal lesions and fructifications develop more rapidly and abundantly, inoculum is more plentiful,

and conditions favoring inoculation and incubation exist. This influence of moisture was strikingly apparent in stands with minor crown openings, where, following a rain, one side of the trunk, remaining wet longer, was frequently observed to bear a continuous series of coalesced lesions and fungal fruiting bodies, while the exposed and rapidly drying side of the same trunk bore but a few delimited circular lesions with a meagre development of fruiting bodies.

Mosses and foliose lichens (see Section VII B, above) were constantly observed to favor the development of the fungus. This was assumed to operate in two ways. The first is by providing an ideal shelter for the wandering nymphs of *Cryptococcus* and the development of feeding colonies of the insect, which soon affords excellent inoculation courts for *Nectria*. The second way is by favoring directly the incubation of inoculum and infection of the suscept through providing anchorage against streams of water which run down the bark during rains, and through maintaining moist conditions long after wind and sun have dried the exposed surfaces of the trunk. The soundness of these views was borne out by examination of countless lichens and mosses. On trees with only minute and occasional insect colonies, larger and older colonies could always be found under mosses and lichens, especially the latter. On a day following heavy rains, when the exposed bark had already dried, moisture could always be found under the thalli. Shaving the bark of recently infested and newly infected trunks beneath 221 lichen thalli disclosed healthy areas under 45.7% of the thalli and necrotic areas under 54.3%; shaving under 230 mosses disclosed healthy areas beneath 31.7% and necrotic areas under 68.3%. On these same trunks the number of necrotic areas per unit of bark surface was far less for exposed bark than for bark covered by lichens and mosses. On recently infected trees the first rings of fructifications usually appear under the lichens, and to a lesser extent the mosses. These statements of observed correlation do not of course constitute proof of a cause-and-effect relation; but, in the opinion of the writer, they strongly suggest an influence.

The size and age of tree seemed, both in general observations and in the permanent sample plots, to be related to the activity of infection, both the amount of necrotic tissue on individual trees and the number of trees killed seeming to vary with size of tree. This is plausible on the grounds that young trees provide less suitable crevices, and support fewer lichens and mosses for harboring the insect and enabling infection, and that potentially more vigorous meristematic tissues are capable of restricting individual infections more successfully and rapidly.

It was decided to test this observation, as well as others with regard to the possible influence of environment on the disease, by including data on these factors with the data gathered from line plots in the survey discussed in Section V.

LINE-PLOT SURVEY

The possible factors examined in the line-plot survey were diameter (d.b.h.) and crown class of the susceptible tree; the stand factors—forest type, percentage of beech in stand, and crown density; the indirect physiographic factors—position on ridge or slope, percentage or angle of slope, aspect (exposure) according to the compass; and the climatic factor—proximity to large bodies of sea water.

Diameter and Crown Class

Size of tree was expressed in this survey in two ways: as diameter (d.b.h., 4.5 feet) and as crown class. When the percentage of beech infected was compared with diameter, no consistent variation in the two was found; the mean percentages of beech infected by *Nectria*, based both on plot-percentage means and on tree totals, for the various four-inch diameter classes were found to range between the narrow limits of 85.0 and 93.5%, and show no correlation with diameter. This result is presumed to signify merely that absolute susceptibility is not influenced by diameter of the trunk. But when the severity of infection of the infected beech (designated by three severity classes: light, medium, and heavy) is compared with diameter, a significant variation is discovered (Table XXI). Examining first the figures for the

TABLE XXI

DISTRIBUTION OF SEVERITY OF INFECTION OF *Nectria*-INFECTED BEECH, BY DIAMETER CLASSES

Region	Diameter by four-inch classes, in.	Number of beech infected by *Nectria*	Per cent of *Nectria* infected beech			Probability of significance of difference in distribution of severity of infection†	
			Light infection	Medium infection	Heavy infection	Between adjacent diameter classes	Between diameter classes 4–7 in. and 12–15 in.
Nova Scotia mainland	4–7	818	9.4	21.8	68.8	>100 to 1	
	8–11	368	5.2	13.8	81.0	Between 19 and 49 to 1	> 100 to 1
	12–15	138	3.6	9.4	87.0	Between 7 to 3 and 1 to 1	
	16–19	33*	0.0	15.2	84.8		
	20–23	3	(Too few to be significant)				
Cape Breton and New Brunswick	4–7	1460	27.3	33.3	39.4	>100 to 1	
	8–11	820	18.8	30.5	50.7	Between 9 and 4 to 1	> 100 to 1
	12–15	343	23.3	26.0	50.7	Between 1 to 1 and 3 to 7	
	16–19	66*	28.8	27.3	43.9		
	20–23	8	(Too few to be significant)				

**Probably based on too few trees to be significant.*

†These values are obtained by computing χ^2 *according to a method in Fisher* (1930: *p.* 84-85, *ex.* 11) *and converting the value of P, secured by entering the* χ^2 *table, into probability odds.*

areas in the Nova Scotia mainland, it appears that, compared with diamet the percentage of infected beech with light infection shows a good negati correlation and the percentage with heavy infection a good positive correl tion. Since these figures are based on tree totals it is not feasible to compu correlation coefficients; another measure of the reliability of this trend afforded by computing the significance of the differences between the distribu tions of the tree percentages (totalling 100%) for the various diameter classes The table shows that probability odds are low for the significance of such differences as are not in agreement with this trend, and high for all which are in agreement. In the Cape Breton and New Brunswick areas, where the disease has not yet run its full course, this trend is not so clear, but is judged to be definite, none the less. The downward trend of light infection and the upward trend of heavy infection are shown in the graph (Fig. 13). In other words, the amount or severity of infection tends to vary directly with the diameter of the trunk.

With the foregoing relation established, one would expect to find a similar relation between mortality and diameter. The mean percentages of beech dead in the sample plots have been secured on the basis both of tree totals and of plot percentages, and are presented in Table XXII. The actual mean for each sample plot is indicated on the scatter diagrams in Figs. 14 and 15, which show that, although scatter is great, the standard errors (indicated by horizontal lines) are small for the lower diameter classes because of the great numbers of points, and that the class means exhibit a definite tendency for mortality to increase with diameter. This trend has been measured by the correlation coefficient (Table XXII) and found, as expected because of the

TABLE XXII

PERCENTAGE OF BEECH DEAD, COMPARED WITH DIAMETER AT BREAST HEIGHT

Region		Diameter by four-inch classes					Correlation coefficient r	Probability of significance of r
		4–7 in.	8–11 in.	12–15 in.	16–19 in.	20–23 in.		
Nova Scotia mainland	Number of beech	906	405	147	35	4		
	Mean per cent of beech dead, based on tree totals	**41.7** ±1.64*	**55.1** ±2.47	**66.0** ±3.91	**60.0** ±8.28	**75.0** ±21.65	—	—
	Mean per cent of beech dead, based on plot percentages	**41.0** ±2.53	**48.3** ±3.73	**67.9** ±4.69	**62.9** ±12.30	**75.0** ±30.96	**0.311** ±0.0612	>100 to 1
Cape Breton and New Brunswick	Number of beech	1600	932	372	74	8		
	Mean per cent of beech dead, based on tree totals	**17.6** ±0.95	**21.4** ±1.34	**25.3** ±2.25	**31.1** ±5.38	**37.5** ±17.12	—	—
	Mean per cent of beech dead, based on plot percentages	**16.3** ±1.73	**19.6** ±1.99	**23.2** ±2.93	**28.8** ±5.54	**42.9** ±20.20	**0.174** ±0.0474	>100 to 1

**The standard error for a mean percentage based on tree totals (such as living and dead) is computed by the formula* $\sigma_Q = \sqrt{\frac{PQ}{n}}$ *where P = per cent living, Q = per cent dead, and n = the total number of trees living and dead.*

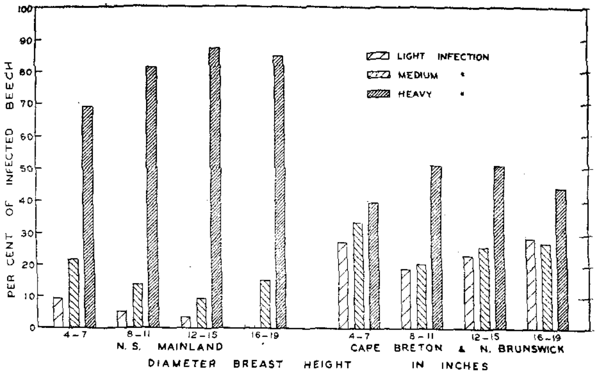

FIG. 13. *Percentages of Nectria-infected beech with light, medium, and heavy infection, by diameter classes; in Nova Scotia mainland, and in New Brunswick and Cape Breton Island.*

scatter, to be small. But the tests of the computed values of r (standard error of r, and probability of significance of the value of r) show that the values secured, though small, are reliable. This in itself does not mean that they

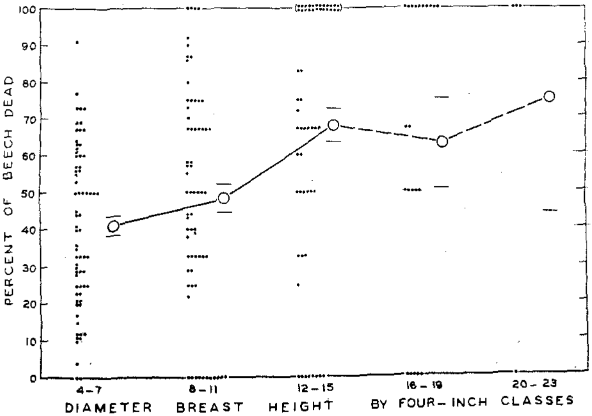

FIG. 14. *Percentage of beech dead (by plots): compared with diameter; for plots in Nova Scotia mainland.*

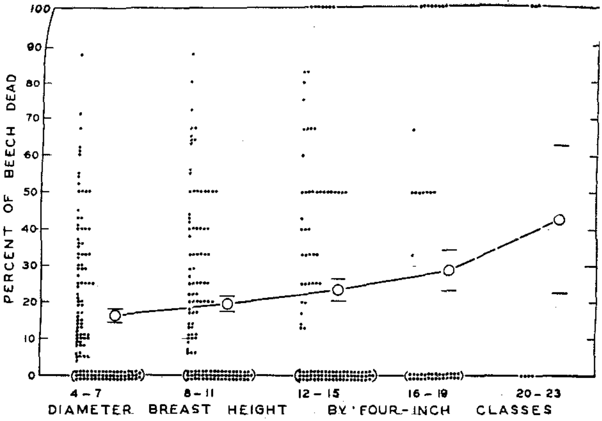

FIG. 15. *Percentage of beech dead (by plots): compared with diameter; for plots in New Brunswick and Cape Breton Island.*

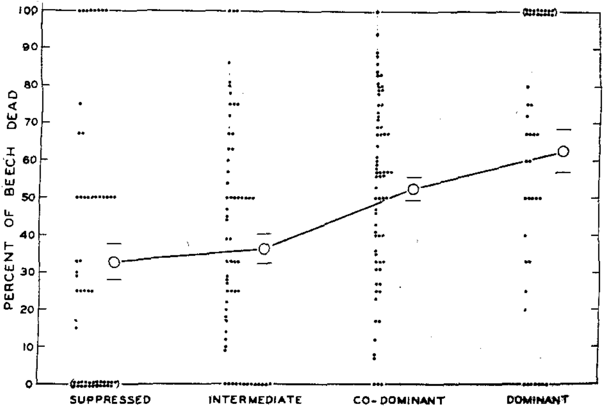

FIG. 16. *Percentage of beech dead (by plots): compared with crown class; for plots in Nova Scotia mainland.*

are significant; the decision as to their significance is largely a matter of judgment. When one considers the innumerable factors (including many of which we are probably not even aware) which must cause local variations both in aggressiveness of the pathogens and resistance of the suscept, the existence of even a small definite tendency for mortality to vary with one of them must be regarded, in the writer's opinion at least, as indication that this factor possibly exerts a definite influence. The question of the nature and biological plausibility of that influence is apart from the foregoing statistical one; it is discussed below.

The other measure of size of the tree employed in this study is crown class. No record was kept of the relation between infection and crown class. The data for mortality have been compiled, as in the case of diameter, on the basis of mean percentages of beech dead according to tree totals and plot percentages, and are presented, with standard errors of the means, in Table XXIII. The scatter of the plot percentages and their means for each crown class are plotted in the scatter diagrams (Figs. 16 and 17), which show, as with diameter, a great scatter, but small standard errors and a definite tendency of the means to vary with crown class. Correlation coefficients computed for the two regions have values of 0.315 for the Nova Scotia mainland and 0.614 for Cape Breton and New Brunswick (as compared with 0.311 and 0.174, respectively, in the case of diameter) and again show satisfactorily small standard errors and high probabilities of reliability. As with diameter, the trend is more pronounced in the case of the Nova Scotia mainland, where

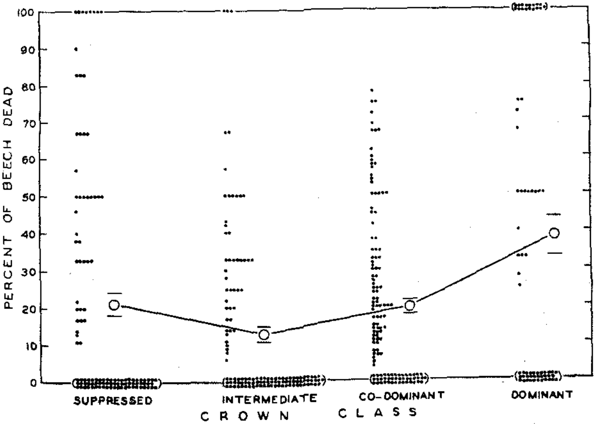

FIG. 17. *Percentage of beech dead (by plots): compared with crown class; for plots in New Brunswick and Cape Breton Island.*

the disease has been active for a relatively long period, than in Cape Breton and New Brunswick.

In short, if one does not object to the reliability of the computed values of r on the ground that the frequency distributions are abnormal, one is faced

TABLE XXIII

PERCENTAGE OF BEECH DEAD, COMPARED WITH CROWN CLASS

Region		Crown classes				Correlation coefficient r	Probability of significance of r
		Suppressed	Intermediate	Co-dominant	Dominant		
Nova Scotia mainland	Number of beech	169	440	772	116		
	Mean per cent of beech dead, based on tree totals	34.9 ±3.67	39.6 ±2.33	54.0 ±1.86	59.5 ±4.56	—	—
	Mean per cent of beech dead, based on plot percentages	32.8 ±4.74	36.6 ±3.92	52.5 ±3.19	62.5 ±5.61	0.315 ±0.05889	>100 to 1
Cape Breton and New Brunswick	Number of beech	428	703	1714	141		
	Mean per cent of beech dead, based on tree totals	22.2 ±2.01	12.4 ±1.24	21.3 ±0.99	39.7 ±4.12	—	—
	Mean per cent of beech dead, based on plot percentages	21.3 ±3.06	12.9 ±1.95	20.9 ±2.01	38.5 ±5.20	0.164 ±0.04731	>100 to 1

with the question of deciding whether the tendency of *(a)* severity of infection and *(b)* mortality to vary with size of tree is significant biologically. As suggested above, this decision is largely a matter of judgment. The writer believes that plausibility comes to the support of *(a)* the relation between infection and size of tree, in that the nature of the bark has been found in extended observations to vary consistently with size of tree, the larger trees having more sheltering lenticular crevices, mosses, and foliose lichens, all of which favor infestation by *Cryptococcus* and infection by *Nectria* in the ways discussed above. Again, the demonstrated relation between *(b)* mortality and size of tree receives support from the following considerations. Let it be assumed, first, that the heavier attack over a more extensive portion of the bark surface on large trees seems to prevent cambial activity from circumscribing and healing-over individual lesions, as frequently occurs on small trees; instead, the radial growth of countless lesions results rapidly in their coalescence and the death of large areas of trunk surface. Second, since the surface area of the crown increases about as the square of the diameter at breast height, it follows that the conducting tissue of the trunk is more active in larger trees because of the greater demands for sap by the proportionately larger crown; and each unit of the conducting system is more vital to the normal activity of the large tree than of the small one. Further, the larger trees are usually co-dominant or dominant, which means that

their crowns are more exposed and therefore subject to greater water loss by transpiration than small trees, which are usually suppressed or intermediate. It follows from these two points that the destruction of a given proportion of the conducting tissues imposes a more severe toll on the normal metabolism and the life of a large tree with exposed crown than on those of a small suppressed tree with sheltered crown.

Stand Factors

Forest type. Although preliminary observations indicated no obvious relation in the Maritime beech stands between the composition of the stand and the amount of infection or mortality, it was believed that such a relation might well exist and, if it did, would offer a valuable silvicultural weapon in control strategy. For the purposes of the present survey, the stands in which beech is common were divided into four arbitrary forest types: (I) *pure beech*, in which over 70% of the stand are hardwoods and 90% or over of the hardwoods are beech; (II) *beech and tolerant hardwoods*, in which over 70% of the stand are tolerant hardwoods (*Fagus grandifolia*, *Betula lutea*, *Acer saccharum*, and others) and less than 90% of the tolerant hardwoods are beech; (III) *beech, other hardwoods, and conifers*, in which not more than 70% of the stand are hardwoods; and (IV) *beech and conifers*, in which not more than 70% of the stand are hardwoods and 90% or over of the hardwoods are beech. Since the actual survey included only a single plot which fell into Type IV, this plot was grouped with Type III. The mean percentages (based on individual plot percentages) of beech infected in all areas were found to be for Type I, 91.2; for Type II, 89.6; for Type III, 88.7. As was to be expected from the probability values in Table III, the mean percentages of beech infected showed only a slight tendency to vary with forest type. The mean percentages of beech dead in the Nova Scotia mainland plots were found to be for Type I, 48.4; for Type II, 48.9; for Type III, 44.5; similar percentages in Cape Breton and New Brunswick proved to be for Type I, 22.2; for Type II, 18.7; for Type III, 18.5. The mortality data, too, thus show no marked correlation with forest type. The figures as a whole show a very slight trend in favor of a positive relation between the proportion of beech in a type and the amount of disease, and a negative one between conifers in a type and disease; but this trend is so small that no statistical analysis has been made and the relation is assumed to be negligible.

Percentage of beech in stand. A better measure of a possible relation between composition and disease is afforded by a direct comparison of the percentage of beech in the stand with mean percentages of beech infected and dead. The plots have been arranged in 10% classes according to the percentage of beech in stand and the actual class means presented in Table XXIV, together with the mean percentage for the plots in each class of beech infected. Here an irregular but positive trend is observed for the mean percentage of beech infected by *Nectria* to vary with percentage of beech in stand. The scatter diagram prepared from the raw data (Fig. 18) shows that the scatter of individual plot percentages is wide, with resulting rather large standard

TABLE XXIV

PERCENTAGE OF BEECH INFECTED BY *Nectria*, COMPARED WITH PERCENTAGE OF BEECH IN STAND, FOR ALL REGIONS

	Per cent of beech in stand, by 10% classes									Correlation coefficient	Probability of significance of r
	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100	r	
Actual mean of per cent-beech-in-stand class	15.0	24.8	33.7	44.0	54.1	64.2	75.2	84.3	93.6	**0.208** ±0.06799	>100 to 1
Mean per cent of beech (based on plot percentages) infected by *Nectria*	**83.3**	**86.9**	**86.4**	**89.1**	**87.7**	**88.8**	**93.3**	**92.6**	**95.9**		
	±5.76	±3.51	±6.24	±2.21	±2.27	±2.03	±1.44	±1.44	±1.12		

errors for the class means. But the trend is definite and the correlation coefficient, although small (0.208), has a low enough standard error and high enough probability odds of reliability to establish the fact of a small positive correlation between percentage of beech infected and percentage of beech in stand. The mortality figures, similarly compiled, show no tendency towards correlation with percentage of beech in stand and the correlation coefficients are negligibly small and unreliable (for plots in the Nova Scotia mainland, 0.00334 ± 0.1204, with probability of significance odds 1 to 9; for plots in Cape Breton and New Brunswick, 0.0513 ± 0.08781, with probability odds between 1 to 1 and 2 to 3). In short, the data show that the percentage of beech infected by *Nectria* shows a small tendency to vary with percentage of beech in stand, but that this relation is not strong enough to exert an appreciable influence on the percentage of beech killed.

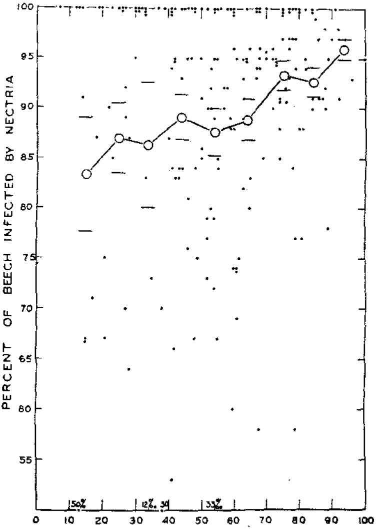

FIG. 18. *Percentage of beech infected by Nectria (by plots): compared with percentage of beech in stand.*

Crown density. Preliminary observations led the writer to believe that stand factors which favor the retention of moisture under the crown canopy promote the activity of the *Nectria* and the virulence of the disease. Among such factors may be included density of the crown canopy and abundance of ground cover and underbrush. Systematic observations on ground cover and underbrush were not made. Notes were recorded in each plot on crown density expressed as tenths, where 0.5 denoted a stand in which 0.5 of the ground was estimated to be shaded and 0.9 denoted approximately 0.9 of the ground shaded, the latter being the greatest crown density which is common in temperate hardwood stands. An important difficulty encountered in estimating crown density, and one which increases with the duration of the disease in a stand, is that the crown density at the time of recording is not the same as it was when the disease began, but decreases progressively; it was therefore necessary to estimate the crown density at the time that the disease

began; it is this figure, designated "past crown density," which is considered here. Computations of mean percentages of beech infected (based on plot percentages) for each past-crown-density class, and dead (based on plot percentages and tree totals), with their standard errors, were made and show that there is no correlation between either percentage of beech infected or percentage dead and crown density. Correlation coefficients were computed for the mortality figures, but proved to be small, less than twice their standard errors, and to have unconvincing probability odds of significance (for the Nova Scotia mainland plots, 0.098 ± 0.1201, with probability odds of significance between 1 to 1 and 2 to 3; for Cape Breton and New Brunswick plots, 0.144 ± 0.0878, with odds between 4 to 1 and 9 to 1).

When severity of infection on the infected beech is compared with crown density, there is seen to be no consistent correlation. In the Nova Scotia mainland the percentage of infected beech with light infection varies directly while the percentage with heavy infection varies inversely with the crown density, a situation the opposite of what was anticipated. In Cape Breton and New Brunswick the situation is more nearly in keeping with expectation, but inconsistent with the Nova Scotia results. The probability odds show that the differences between crown-density distributions of the severity of infection are in nearly all instances greater than can be accounted for by chance. The explanation of these results is not clear; they indicate that some factor or factors other than crown density are operating more effectively.

Indirect Physiographic Factors

Since most of the beech in Nova Scotia and New Brunswick occurs on hardwood ridges, data on three indirect physiographic factors which were thought to have a possible connection with the disease were recorded. These were position of plot on ridge, degree of slope, and aspect.

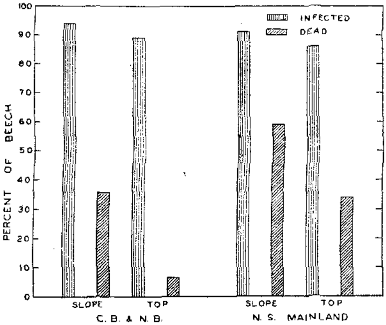

FIG. 19. *Mean percentage of beech infected by Nectria, and dead: compared with position on ridge; for plots in Nova Scotia mainland, and in New Brunswick and Cape Breton Island.*

Position on ridge. It had been noted in preliminary observations that the beech on the slope (or face) of a ridge were killed in greater number than those on the broad top. Since conditions are usually more conducive to drying on the face, owing to greater crown exposure and air currents, it was thought that greater mortality was due less to increased activity of the pathogens than to decreased ability of the tree to withstand an attack of given intensity—for the same reasons discussed under

TABLE XXV

PERCENTAGE OF BEECH INFECTED BY *Nectria*, AND PERCENTAGE DEAD, COMPARED WITH POSITION ON RIDGE

Region		Nova Scotia mainland		Cape Breton and New Brunswick	
Position on ridge		Slope	Top	Slope	Top
Total number of beech		632	438	941	941
Per cent of beech INFECTED, based on	Plot percentages	**90.8**	**85.6**	**94.2**	**88.8**
	Tree totals	**92.1** ±1.07	**87.0** ±1.61	**94.8** ±2.29	**87.4** ±1.08
Difference between slope and top percentages		5.1 ±1.93*		7.4 ±2.53	
Probability of significance of difference†		>100 to 1		>100 to 1	
Mean per cent of beech infected, all regions combined		89.6 ±0.77			
Per cent of beech DEAD, based on	Plot percentages	**59.0**	**35.6**	**34.8**	**6.59**
	Tree totals	**60.9** ±1.94	**34.0** ±2.26	**34.4** ±1.55	**7.01** ±0.83
Difference between slope and top percentages		26.9 ±2.98*		27.4 ±1.76	
Probability of significance of difference†		much >100 to 1		much >100 to 1	
Mean per cent of beech dead, by regions		48.0 ±3.5		19.2 ±1.7	

**The standard error of the difference between two means is computed by the usual formula:* $SE_{m_1-m_2}=\sqrt{SE^2_{m_1}+SE^2_{m_2}}$. *A significant difference between two means should have a standard error of less than one-half of the difference.*

†*These values are odds, and measure the probability that the difference between the contrasted series could not have arisen by chance. They are obtained by computing the constant* χ^2 *according to the formula for "fourfold" tables in Fisher (*1930: *p.* 84*) and converting into probability odds the value of P obtained by entering the* χ^2 *table.*

The odds are found to be more than 100 *to* 1 *that the differences both in mortality and in infection between slopes and tops of ridges are not chance results. In other words, the differences are significant.*

crown class (above). In order to discover whether infection and mortality actually vary with position on ridge, a selection from all the plots was made taking only those which were definitely ridge top and those distinctly ridge slope. The results of this selection and of analysis of the data are presented in Table XXV. It will be observed that, contrary to expectation, there is for both regions a slight but significant difference in the percentage of beech infected, being greater on the slope than on the top. The percentages of beech dead show, as expected, a great difference, as between slope of ridge and top. These differences are shown graphically in Fig. 19. A satisfactory explanation of the infection figures is not at hand, but the writer is confident that the difference in the mortality figures is greater than can be accounted

for on the basis of the infection figures, and believes that the explanation lies in the fact that the crowns on the slope of a ridge are more exposed to drying, and that therefore a given amount of necrotic tissue would exert a more lethal influence on such trees than on ridge-top individuals.

Percentage of slope. Another measure of the same influence was thought to be afforded by steepness of slope. The plots were sorted according to slope and the percentages of beech infected and dead compiled for the different slope classes. The mean percentages of beech infected are exhibited for 10% slope classes (100% slope equalling an angle of 45 degrees) in Table XXVI and show a small and questionable correlation of infection with slope. The mortality data were analyzed similarly and showed for the Nova Scotia mainland plots an r value of 0.192 ± 0.1160, with probability odds between 9 and 19 to 1; the Cape Breton and New Brunswick percentages were so erratic that r was not computed. Thus there is no correlation of mortality with slope. These results suggest that the foregoing explanation of the influence of exposure on death may not be correct—or at least not sufficient.

Aspect. Believing that insolation, prevailing winds, and other factors might exert an influence on the disease, it was decided to compare infection and mortality with aspect (compass direction with the slope faces), since aspect affords a ready index to such factors. The results (Table XXVII) show

TABLE XXVII

PERCENTAGE OF BEECH INFECTED BY *Nectria*, AND PERCENTAGE DEAD, COMPARED WITH ASPECT

Mean per cent of beech (based on plot percentages)	Region	Aspect (described in eight points of the compass)							
		N	*NE*	*E*	*SE*	*S*	*SW*	*W*	*NW*
Infected by *Nectria*	Nova Scotia, Cape Breton, and New Brunswick	91.1	85.9	85.0	90.0	85.4	88.3	93.8	89.9
Dead	Nova Scotia mainland	47.5	52.4	54.6	43.2	42.6	30.0	45.7	47.0
	Cape Breton and New Brunswick	32.7	14.7	10.8	14.8	24.1	9.7	21.8	19.2

no consistent variation either of infection or of mortality with aspect. The apparently low mortality on the southwest aspect is perhaps explained in part by the fact that only a very few plots had this aspect. It is possible that the southwest aspect is unfavorable to beech; but whether such a condition would increase or decrease resistance to infection is open to question. Little significance can be attached to the greater variability in the Cape Breton and New Brunswick figures, since the disease is still relatively young in these regions.

TABLE XXVI

PERCENTAGE OF BEECH INFECTED BY *Nectria*, COMPARED WITH PERCENTAGE SLOPE OF GROUND, FOR ALL REGIONS

	Per cent slope of ground, in 10% classes*									Correlation coefficient	Probability of significance
	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	90–99	*r*	of *r*
Actual mean of per cent-slope class	2.6	12.4	21.0	30.4	40.0	50.0	60.0	72.5	100.0		
Mean per cent of beech (based on plot percentages) infected by *Nectria*	**87.4**	**90.9**	**89.2**	**90.3**	**93.4**	**93.9**	**90.8**	**91.7**	**94.2**	**0.164** ±0.0693	between 49 and 99 to 1
	±1.57	±1.82	±2.62	±1.87	±3.05	±1.65	±6.13	±3.00	±0.91		

*100% *slope* = 45° *angle of slope.*

Climate

Proximity to sea water was the only climatic factor examined for its possible influence on infection and mortality. The plots were arranged according to their distance by 10-mile classes from the nearest large body of salt water, and the means, with their standard errors, computed of the plot percentages of beech infected and dead in each 10-mile class. The results as a whole showed no consistent trend and appear inconclusive. In the first two classes (0–9, and 10–19 miles from the sea), however, both infection and mortality figures for the Cape Breton and New Brunswick plots decreased with distance from the sea, while in the Nova Scotia mainland plots both figures increased. Whether this result has significance is not clear. It was thought from preliminary observations that fogs along the coasts, which seem to account in part for the abundance of lichens and mosses in the Maritime forests and to favor the proverbially rapid reproduction of the softwoods, might also stimulate the activity of the *Nectria*. The present comparisons can hardly be regarded as conclusive evidence that no such influence exists; but they indicate that it is not pronounced.

SUMMARY

Preliminary observations and a line-plot survey in Nova Scotia and New Brunswick have shown that while the amount of infection is almost uniformly great in the areas affected, the mortality varies in different localities but is generally related to the duration of the local outbreaks. Furthermore, it has been shown that local variations in both infection and mortality are correlated with certain differences in size of tree, nature of stand, and physiography. Small but definite positive correlations exist between percentage of beech infected by *Nectria*, and percentage of beech in stand, position on slope of ridge, and steepness of slope; further, severity of infection shows definite correlation with diameter (d.b.h.), and questionable correlations with forest type and crown density. Mortality, expressed as percentage of beech (over 3 in. d.b.h.) killed, is correlated definitely with diameter, crown class, and position on slope of ridge; and questionably with forest type, and steepness of slope.

In no case are the correlations high. Although this fact might on first thought tend to weaken one's confidence in the results, the writer believes that the existence of even a small determinable correlation, when statistically reliable, has great meaning because it indicates a trend so strong that it stands out above the many other influences which must be exerted locally both on the pathogens and on the suscept. The mere existence of correlation between two variables is no proof that one causes or even influences the other. But if from other sources one has an understanding of the nature of the relation between two variables which have been found statistically to move together, then correlation coefficients and other statistical devices are useful in affording a reliable estimate or measure of the magnitude of the relation. Since the computed correlations in the foregoing data are in accord with

biological facts or theories, they are judged by the writer to have significance in the present study. The question of their applicability to control practice is apart from the present discussion and will be dealt with below.

IX. Control

The problem of control of the beech bark disease demands consideration from two aspects, *(a)* that of a favorite ornamental shade tree, and *(b)* that of one of the less valuable of the timber producing hardwoods at present. Since fungal infection is dependent on previous attack by the woolly beech scale, direct control of the insect may be regarded as one means of preventing the development of the disease. That direct control of the fungus would be difficult, if possible, is suggested by experience with other fungal bark diseases of trees. Indirect means, because of the necessity for relatively small expenditures, are more favored in forest-disease control than the costly direct means, applicable to ornamental trees. The direct means which are discussed below by reason of their applicability to ornamental beech are (1) exclusion from regions at some distance from infested areas by nursery inspection, and (2) protection or early eradication in exposed or newly infested areas. The possible indirect means which may find employment in forest control are (1) the discovery and cultivation of fungal parasites or insect predators or parasites of *Cryptococcus*, and (2) the practice of silvicultural measures designed to decrease the aggressiveness and virulence of the pathogens. An added economic measure of great importance in severely attacked stands is the timely salvage of the timber that has succumbed.

A. CONTROL ON ORNAMENTAL BEECH

Exclusion

One of the most satisfactory means of controlling a biotic disease is the prevention wherever possible of the distribution into uninfested regions of the pathogens that cause the disease. In the case of the beech bark disease, nursery inspection and prohibition of the transportation of nursery stock or other materials likely to harbor the pathogens would, with a high degree of certainty, prevent the disease from spreading to regions isolated by large natural barriers. The exercise of such measures within a continuous area would at best be of variable worth as a retarding factor, their value depending on the distances involved, the direction of prevailing winds, and the—as yet undetermined—natural distribution of the fungal pathogen. Climate, as a limiting factor of spread, applicable to both beech ornamentals and forests, may be an operating influence of great importance; but on this subject no information has yet been assembled.

Protection and Eradication

The writer has made no experiment on the direct protection from fungal infection of beech exposed to such infection in the regions harboring the fungus, by spraying for the insect; but the protection experiments described in Section VII C, D, indicate that when trees are protected against infestation

or disinfested within a year of attack, fungal infection does not generall follow. Direct control of *Cryptococcus fagi*, by protecting trees exposed t infestation, and eradicating the insect from infested trees by the employmen of insecticides, can be readily and effectively practised. The references tc this insect in Europe abound with recommendations and instructions for scrubbing and spraying susceptible portions of the tree with all manner of contact insecticides. Complete success is reported to attend their thorough application. The most highly endorsed materials in recent times are a German proprietary insecticide called "carbolineum" and various English mixtures whose active ingredients always include one of the coal tar hydrocarbons.

The writer has investigated the problem particularly with respect to New England conditions, and the results have been satisfactory (Ehrlich, 1932 a). There seems no reason to suppose that the successful measures employed would not be equally effective in other areas. With a view to selecting an easily obtainable and effective insecticide for New England use, preliminary tests were made in the late winter of 1931 with various strengths of commercial Sunoco oil, homemade kerosene-soap emulsion, nicotine sulphate (Black Leaf 40), and lime-sulphur. The tests were made by soaking an infested area of bark with the aid of a hand spray gun and removing samples of the bark at once and at intervals of several days for microscopic examination. The efficacy of the various materials was determined by placing the bark on the stage of a binocular microscope and gently raising individual nymphs from the bark with a needle so as not to injure them while thus forcibly withdrawing their stylets from the bark. They were then rolled over so that their ventral sides were uppermost. Those not killed by the insecticide indicated their vitality by waving the stylets above their bodies. Others, raised slightly, but not sufficiently to cause complete withdrawal of the stylets from the bark, waved their bodies about, pivoted only on the stylets. A sufficient number of nymphs were examined in this way from every bark sample so as to leave no doubt as to the effect of a particular treatment. The results of these preliminary tests indicated that lime-sulphur and nicotine sulphate are not satisfactory materials but that Sunoco oil and kerosene-soap emulsion are suitable.

Later in the spring of 1931, field tests were made with commercial equipment operated by park employees in the Arnold Arboretum, Boston parks, and the Middlesex Fells Reservation, using Sunoco oil, kerosene-soap emulsion, and nicotine sulphate. These applications were repeated in the spring of 1932. The pertinent results of these tests are brought together in Table XXVIII. It was concluded from these results that Sunoco oil, 1 to 15, is the most satisfactory material. The critical factor in these tests seemed to be the ability of the operator to cover the entire surface of the tree with sufficient thoroughness so that the material wet the fluffy canopy protecting the insects and penetrated to their bodies. This was possible only with the oils. A few trees were also scrubbed with each of these materials, using a long pole with a scrub brush screwed to one end. It was found that this method, when used

TABLE XXVIII

FIELD TESTS WITH CONTACT INSECTICIDES FOR CONTROL OF THE BEECH SCALE

Material	Strength	Place	Number of infested trees sprayed	Number of sprayed trees scale-free*		Per cent control	
				1931	1932	1931	1932
Sunoco oil	1 to 15 of water	Arnold Arboretum	15	15	14	100	93
Kerosene-soap emulsion	25%	Willow Pond Road, Boston	18	12	17	67	95
Sunoco oil, soap, and Black Leaf 40	1 to 10 of water ⅓ pt. per 40 gal.	Middlesex Fells	69	69	69	100	100
Water Black Leaf 40, and soap	50 gal. ⅓ pt. 2½ lb.	Jamaica Pond, Boston	9	0	8	0	89†

**Number of these trees on which no living scale could be found on careful examination of accessible parts with a hand-lens one month after spraying.*

†*Sunoco oil applied with this material in 1932.*

with Sunoco oil or kerosene-soap emulsion, was much the most thorough. But it is a difficult method and the labor and expense involved make it applicable only in estates where a small number of valued shade trees are to be kept completely free of the scale.

B. CONTROL IN THE FOREST

Fungal and Insect Parasites

A control measure of technical appeal to the investigator but as yet of uncommon employment by the practical entomologist, horticulturist, and forester is the cultivation of natural enemies of the noxious insect. These include fungi and other insects. Neither in the literature nor in the personal observations of the writer has a fungus parasitic on *Cryptococcus fagi* been encountered. Yet it is believed that watchfulness should not be relaxed, because the environmental conditions favorable to the development of this insect are those commonly suited to fungal activity. For this reason a parasitic fungus, once liberated in an infested region, might well thrive and result in a material diminution of the insect.

Parasitic or predacious insects associated with *Cryptococcus fagi* have occasionally been reported in the literature but none were carefully observed or accorded significance. As early as 1849, Baerensprung stated merely that "Zwischen den Larven lebte eine schwefelgelbe, stark behaarte Milbe." In the following year Hardy (1850 : p. 70) noted: "I must also defer to another opportunity an account of a minute mite, as well as a caterpillar, that accompanied, apparently as parasites, the specimens that have given rise to the remarks now offered"; so far as the present writer is aware, another opportunity never came. Newstead (1901 : p. 28-29) observed a lace-wing fly (*Chrysopa* sp.) on beech trunks infested with *C. fagi* but never found the larvae feeding

on the beech scale. Theobald (1905 : p. 137) stated: "So far no parasite of any importance has been found, but some specimens . . . . had quite a number of larval lady-birds among the felted mass of Coccids. They did not appear ravenous, however, and made little headway against the Coccus." Other observers, including Rhumbler (1915) in Germany, Trägårdh (1921) in Sweden, and an anonymous member of the Forestry Commission (1926) in Great Britain, stated definitely that no parasitic or predacious insects had been found. In Nova Scotia, however, Swaine (1930 : p. 45) reported "an unusual abundance of native Coccinelid beetles, which prey on the coccus." The writer made consistent observations in 1930, 1931, and 1932 on the presence of *Chilocorus bivulnerus* Muls. on *Cryptococcus*-infested beech in seven of the permanent sample plots in Nova Scotia, the results of which are summarized in Table XXIX. This coccinelid was commonly observed feeding

TABLE XXIX

Percentage of beech in permanent sample plots harboring *Chilocorus bivulnerus* Muls. (1930, 1931, 1932)

Plot	1930				1931				1932			
	Larvac		Adults		Larvae		Adults		Larvae		Adults	
	Few	Many	Few	Many	Few	Many	Few	Many	Few	Many	Few	Many
1	19	3.4	5.4	0.3	6.5	—	8.2	—	1.4	2.0	1.4	—
2	—	—	—	—	1.4	—	6.9	—	0.4	0.4	—	—
3	—	—	—	—	4.1	—	—	—	4.1	—	—	—
4	—	—	14	—	16	—	4.9	—	0.6	—	2.4	—
5	—	—	—	—	—	—	1.8	—	0.8	—	0.8	—
6	—	—	43	5.4	20	—	13	—	11	1.8	1.8	—
7	—	—	25	4.8	12	—	14	—	1.4	2.0	2.7	—

on the woolly beech scale, but was never found in numbers sufficiently large to exert appreciable curtailment on *Cryptococcus* infestation. Many other species of insects and several arachnids were found to be common on the scale-infested trees but none of them were seen feeding on *Cryptococcus*.

Silviculture

Forestry in America is now passing from the juvenile stage of its progress to more stable and permanent silvicultural practices leading towards sustained yield. Although present economic conditions will retard the profitable employment of these practices, they are bound to occupy an increasingly important place in forest management. When disease control in the forest can be effected in conjunction with such methods, control is most likely to be attempted and offers probably the most useful means of combating many widespread diseases. The studies reported above on the beech bark disease indicate that incidence of infection and mortality vary sufficiently with certain regularizable conditions to hold promise of effecting through them a profitable curtailment in the losses resulting from this disease. Percentage

or severity of infection was found to vary with percentage of beech in stand, steepness of slope, position on top or slope of ridge, and d.b.h., and questionably with crown density. Mortality was discovered to be correlated with d.b.h., crown class, and position on top or slope of ridge. These variations suggest two practices which could well be incorporated in silvicultural operations and whose effectiveness should be tested in sample plots under regulation. Greater infection and mortality associated with position of beech on steep slopes of hardwood ridges indicate that beech should be removed from the slopes and favored on the broad tops of hardwood ridges. The correlation with size of tree, percentage of beech in stand, and crown density suggest that thinning operations should be attempted. Such thinning would have three objectives. First, it would be designed to produce many minor crown openings; this condition should result in decreased abundance and severity of attack by the pathogens, and in retarded activity of those already present. Second, it would remove more beech than other species, in order to lower subsequent infection of beech by decreasing the proportion of beech to other species in the stand. Third, it would cull the trees of larger diameter and higher crown class; this would serve *(a)* to remove the more susceptible individuals most likely, in affected regions, to be already infected and dying; *(b)* it would salvage those less likely than the smaller trees to recover from attack by the fungus; *(c)* it would leave a less susceptible stand capable of producing after a period of years a valuable crop of sound timber; and *(d)* it would supply immediate revenue that might more than pay for the cost of the operation.

Salvage

Dying and dead trees, especially those on which large areas of killed bark adhere and form a substratum for a variety of insects and fungi, become rapidly unfit for merchantable timber by decay of the sapwood. Where the bark loosens and scales off, the exposed areas of sapwood dry and split and in wet weather decay both on the surface and along the fissures. These changes begin on many trees a year before complete death, with the result that when such trees finally die their boles are already more or less deteriorated.

In the Maritime areas where the disease is still active and causing the death of many trees, cutting and marketing of the infected, dying, and dead trees is an urgent necessity if there is to be any salvage from the severely attacked stands. In the newly attacked areas of New Brunswick and Maine, the recently infected stands should be cut before the still-sound timber becomes greatly depreciated in value through splitting and decay.

Salvage operations in attacked stands are recommended, not only because of their immediate economic justification, but also because they constitute important sanitary measures. The various secondary insects and fungi which thrive on decaying slash multiply so prolifically that they soon become an increasing danger to the remaining stand, taking advantage of every wound or weakness resulting from unfavorable soil or weather conditions, attacking and depreciating timber which under normal conditions would maintain a thrifty healthy growth.

In short, control of the beech bark disease in forest stands of the affected and exposed regions should look, first, to salvage of the infected, dying, and dead timber for the purposes of securing the greatest possible return on a rapidly depreciating investment and of preventing the development of unsanitary conditions conducive to further deterioration in the remaining stand. Control should look, secondly, to the possibility of combating the insect pathogen with fungal and insect enemies; and, thirdly, to altering the nature of the stand by forest management designed to minimize the activities of the pathogens and at the same time to substitute a younger, less susceptible stand.

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Explanation of Plates

PLATE I

Beech ridge in Inverness County, Nova Scotia, affected by the beech bark disease. The trees with light foliage (pale green or yellow) were infected recently, those with dark foliage died during the current growing season, and those with naked crowns died during the previous year.

PLATE II

A. Trunk of ornamental *Fagus sylvatica*, lightly infested with *Cryptococcus fagi;* Boston, Massachusetts. B. Trunk of forest *Fagus grandifolia*, heavily infested with *Cryptococcus fagi;* Inverness County, Nova Scotia. C. Heavy two years' infestation, showing woolly wax secretion of *Cryptococcus fagi* (× ¾). D. Heavy three years' infestation, showing woolly wax secretion of *Cryptococcus fagi* (× ¾). E. Light two and three years' infestation with *Cryptococcus fagi*, showing aggregation in lenticular crevices and extrusion of "wool" (× 6). F. Same as Figure E (× 33).

PLATE III

A. First indication of infection; circular lesion, showing ring of macroconidial pustules (× 2). B. Detail of macroconidial pustules, showing erumpent spore masses and upturned ruptured phellem (× 15). C. Circular lesion, showing clusters of perithecia occupying former macroconidial pustules (× 2 to 3). D. Clusters of maturing perithecia, still surrounded by upturned phellar margins (× 15). E. Clusters of mature perithecia, raised above bark and crowding on obscured stromata; ascospore masses beginning to plug ostioles (× 15). F. Clusters of perithecia, collapsed following discharge of ascospores (× 15).

PLATE IV

Same as Plate III, Fig. E (× 33).

PLATE V

A. Cracking bark of dying old beech, showing outlines of original circular lesions. B. Early symptoms of infection: coalescing circular lesions slightly depressed by death of tissues, fruiting pustules occupying central portions; and incipient cracking of bark. C. Exposed young tree, showing ruptured, raised, and sunken localized lesions, and remnants of *Cryptococcus* infestation still active. D. Trunk of young exposed beech, showing old circular lesions under dead foliose lichens, now depressed by surrounding cambial activity; showing also *Cryptococcus* colonies on inner edge of tender marginal callus. E. Two to four years' infected young trunk, showing recent, pustulate, circular lesions, and gnarled and pitted surface resulting from old lesions. F. Long infected deeply pitted young trunk, with fresh *Cryptococcus* colonies developing again on callus.

PLATE VI

A, B, C. Surface and progressively deeper plane views of portion of young, infected trunk, showing two cracked and depressed lesions surrounded by callus, and extent of old and advancing necrosis in the wood. D. Area of trunk with bark freshly removed, showing penetration of circular lesion to wood. E. Beech killed by *Nectria*, dead two years, showing cracked and loosening bark with darkened pustulate areas, and split and discolored wood. F. Mixed hardwood stand on broad ridge-top, Annapolis County, Nova Scotia, showing all beech killed; maple and birch in leaf. G. Roadside view of mixed hardwood stand, Cumberland County, Nova Scotia, showing all beech killed; maple only in leaf.

PLATE VII

A. Stylet track of *Cryptococcus fagi* following anticlinal intercellular course part way through sclerenchyma band in bark of *Fagus grandifolia*. B. Transverse section through lenticel, showing *Cryptococcus* nymphs nestling under phellar shelves at margins. C. Style track passing intracellularly through pericycle parenchyma and deflected horizontally (periclinally) at edge of sclerenchyma band. D. Stylet tracks passing intracellularly through pericycle parenchyma, showing thickening of sheath material in cell lumina. E. Stylet track passing straight through pericycle, not deflected by sclerenchyma. F. Suctorial apparatus of *Cryptococcus fagi*, including salivary glands, rostrum, and stylets passing through periderm. G. Stylet track passing through periderm and cortex.

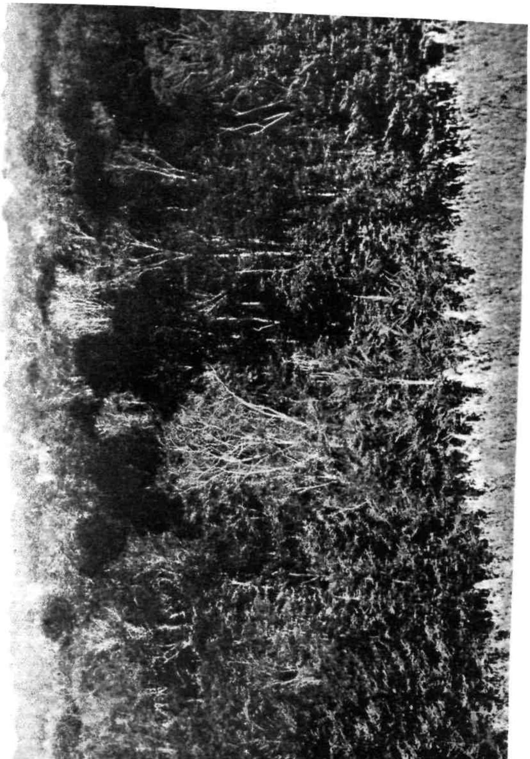
PLATE VIII

A, B. Young stroma of *Nectria* raising and bursting periderm in bark of *Fagus grandifolia*. C. *Nectria* stroma erumpent in bark, bearing macroconidia *(Cylindrocarpon)*. D. Same as Fig. A. E. Same as Fig. C. F. *Nectria* stroma bearing mature perithecia.

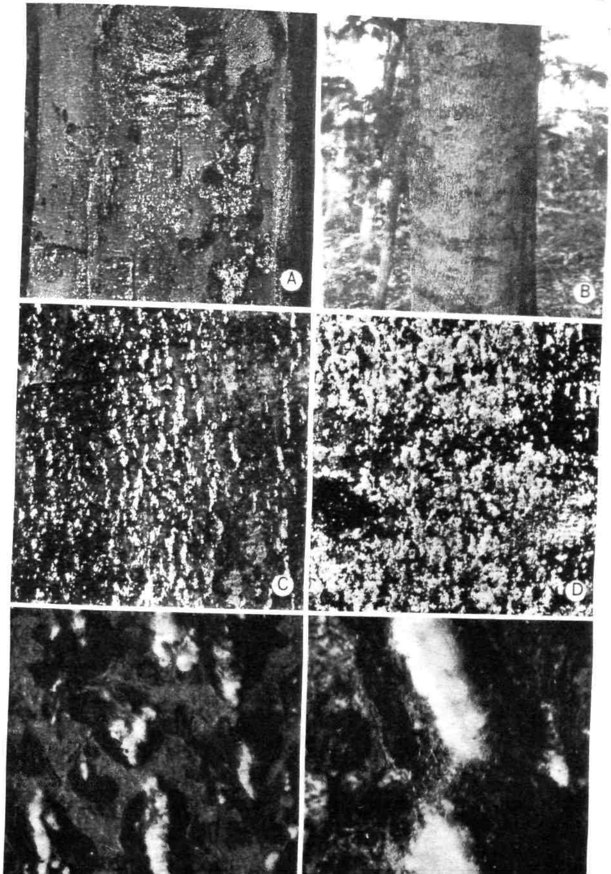
PLATE IX

A. Fresh macroconidial pionnotes of *Nectria* in culture on potato dextrose agar slant (× 6). B. Old dried macroconidial pionnote of *Nectria* in culture on potato dextrose agar slant (× 6). C. Mature *Nectria* perithecia in culture on drying potato agar Petri plate, showing columns of ascospores discharged under moist conditions (× 33). D. Moist chamber tent used for greenhouse inoculations. E. Wood surface of artificially wounded and bark-inoculated beech, showing infected areas. F. Another method of maintaining moisture for greenhouse inoculations of potted sapling beech; absorbent cotton wick carries water from pail to trunk, water seeps down trunk and keeps moist the cotton collars sealing celluloid inoculation chambers. G. Detail of portion of celluloid inoculation chamber, showing bottom sealed by collar of wet absorbent cotton, moist bark within chamber, and signs of successful inoculation. H. Beech trunk, showing method of protecting uninfested and disinfested areas of bark against subsequent attack by wrapping in tarred factory cotton. I. Beech trunk, showing method of protecting uninfested and disinfested areas of bark against subsequent insect attack, but not against fungal inoculation with air-borne spores, by covering with fine mesh phosphor-bronze wire screening raised from the bark and sealed at points of contact with "Tanglefoot." J. Surface of bark with periderm removed on trunk of experimental beech, showing method of examining protected areas and adjacent unprotected areas (above) for symptoms of *Nectria* infection; many lesions in unprotected area; few in protected area.

PLATE I

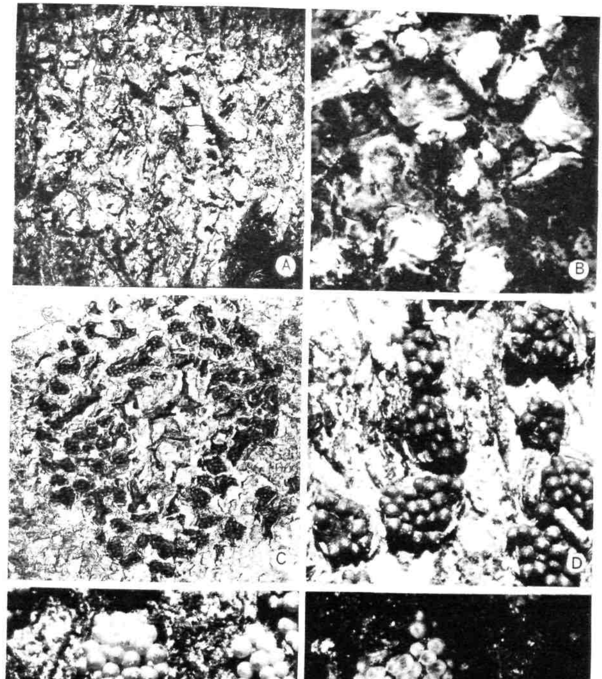


BEECH BARK DISEASE



BEECH BARK DISEASE

PLATE III



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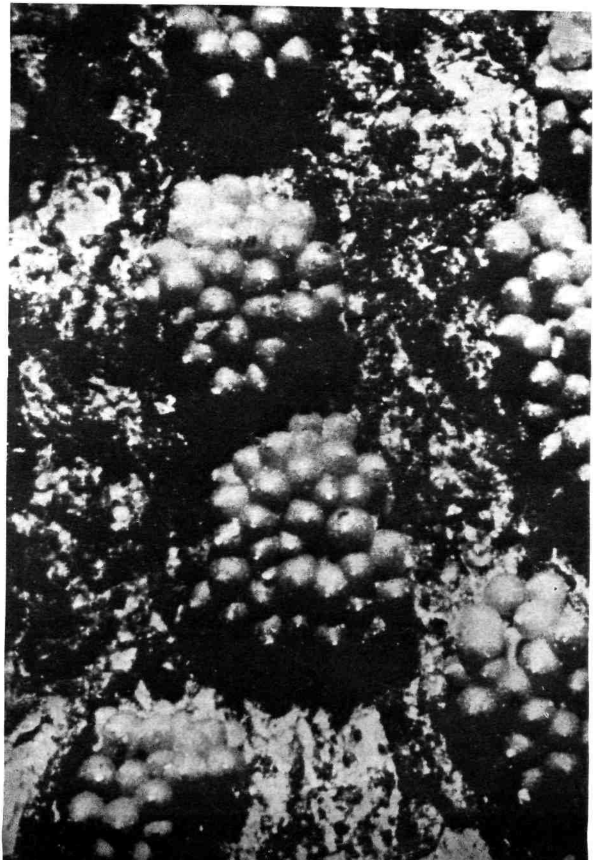
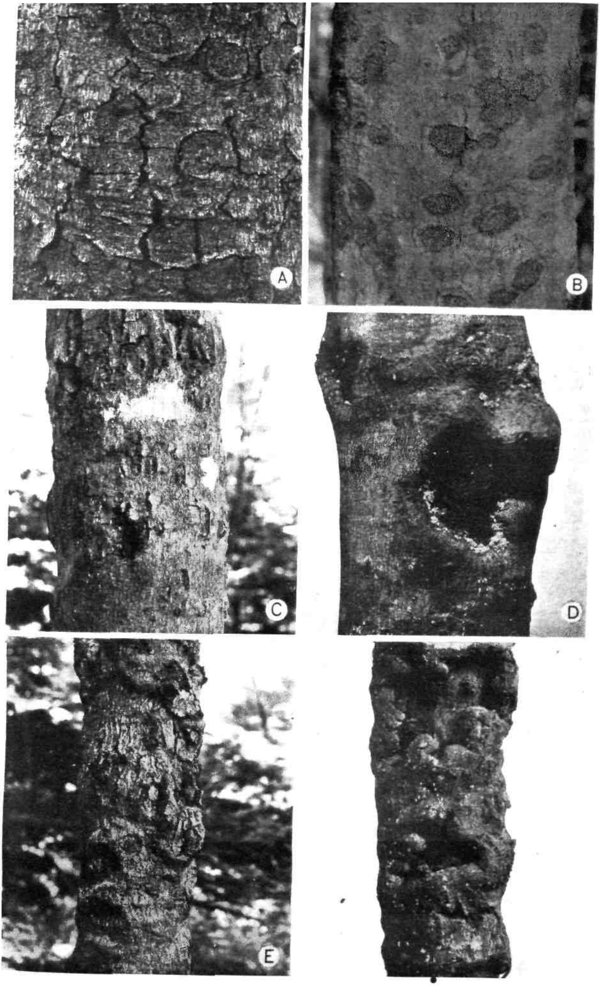


Beech Bark Disease



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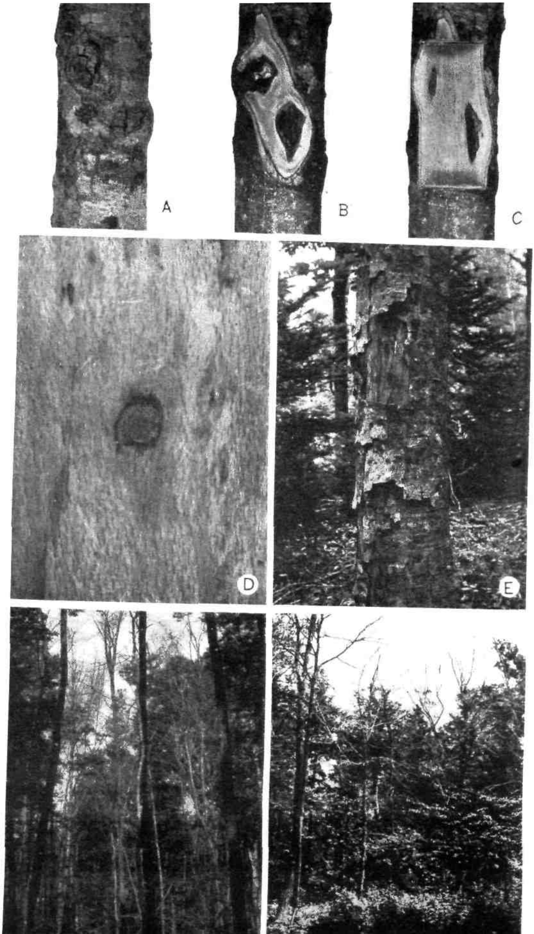

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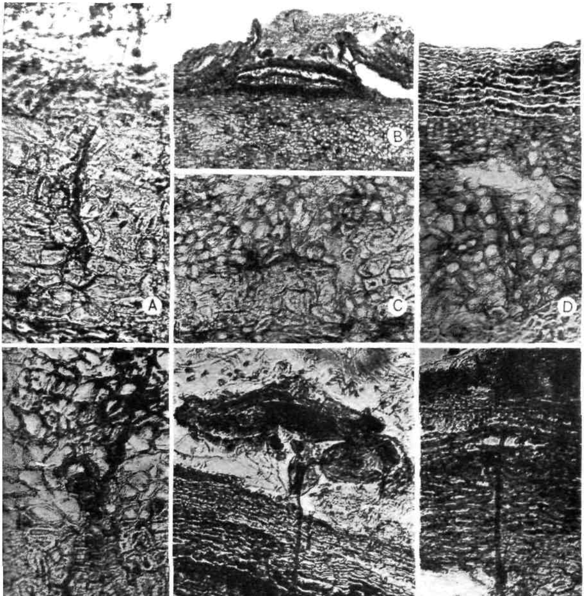

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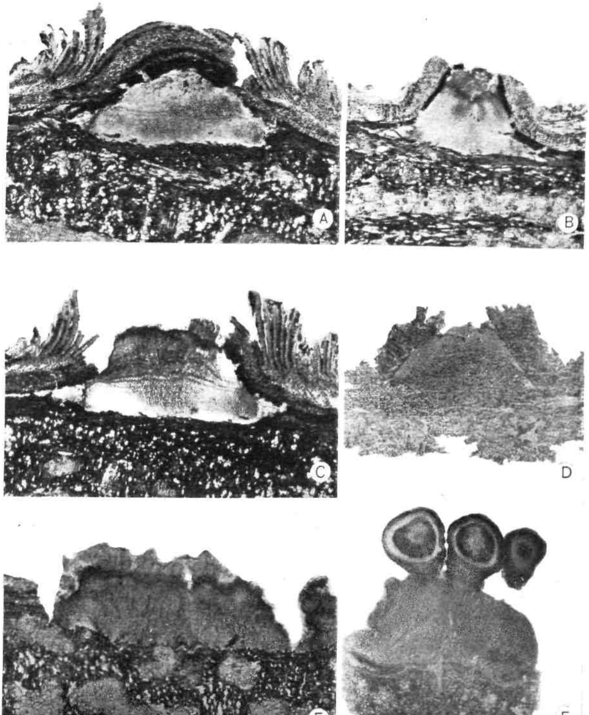

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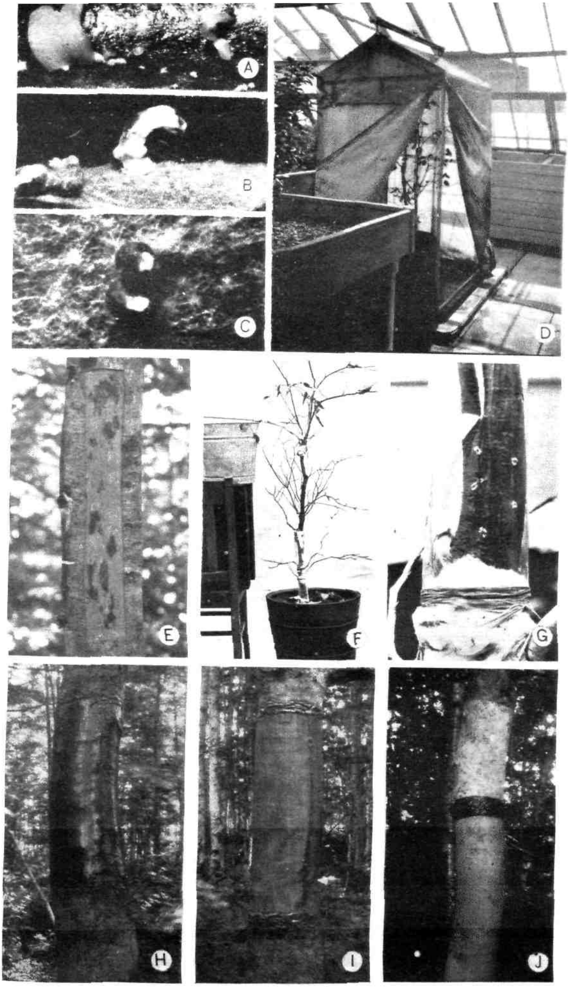

BEECH BARK DISEASE

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NUCLEAR MOMENTS OF ANTIMONY ISOTOPES—DISCUSSION OF LANDÉ'S THEORY[1]

BY M. F. CRAWFORD[2] AND S. BATESON[3]

Abstract

Hyperfine structures in the Sb IV spectrum have been investigated. Analysis of the hyperfine patterns shows that the I-values of Sb_{121} and Sb_{123} are $\frac{5}{2}$ and $\frac{7}{2}$ respectively, in agreement with those deduced by Badami from Sb II. The magnetic moments of Sb_{121} and Sb_{123}, calculated from the hyperfine separations in Sb IV, are 4.0 and 3.2 proton magnetons respectively. For antimony the ratio of the $g(I)$ factors is $g(I)_{121}/g(I)_{123} = 1.82 \pm .02$. The application of the Landé $g(I)$ formula to this accurate ratio leads to a value $g_s = 3$ for the magnetic factor of the proton spin, instead of $g_s = 4$ as found by Landé. The value $g_s = 3$ gives slightly better general agreement between the observed and the calculated $g(I)$ factors than is obtained for $g_s = 4$. However, since no specific value of g_s will satisfy the accurate ratio criterion for all the Z_{odd}, M_{odd} isotopes to which the test can be applied, it is concluded that the theory is incomplete.

Introduction

A spark spectrum involving configurations with an unpaired *s* electron is particularly suitable for hyperfine structure analysis. The high effective nuclear charge in the ion and the large nuclear interaction of the penetrating *s* electron are effective in producing wide magnetic hyperfine separations. The writers' experience with a number of spectra of this type (5, 16, 17, 18*, 19) suggested investigating Sb IV (2, 8) to determine the nuclear moments of the antimony isotopes (15). A cursory examination of the antimony spectrum in the region 3800Å to 2500Å with a three-metre concave grating and a multiple prism spectrograph showed that a number of Sb IV lines have wide, complex structures. The patterns, however, were not sufficiently resolved to be interpreted uniquely. Accordingly the investigation was continued using an instrument of higher dispersion and resolving power. The results obtained are presented and interpreted in this paper. During the course of the investigation Badami (3, 4)† published results on hyperfine structures in Sb I and Sb II, and from his data deduced the I-values $\frac{5}{2}$ and $\frac{7}{2}$ for Sb_{121} and Sb_{123} respectively. These values are in agreement with the values determined by the writers from the structures in Sb IV.

[1] *Manuscript received April 9, 1934.*
Contribution from the Physical Laboratories, University of Toronto, Toronto, Canada.
[2] *Lecturer, University of Toronto, Toronto, Canada.*
[3] *Holder of a fellowship under the National Research Council of Canada.*
* *Also unpublished data.*
† *See also Murakawa (20).*

Experimental

The lines were excited by an electrodeless discharge in antimony vapor (16). Under the proper experimental conditions this type of source emits lines of high excitation with good intensity. Further the line components show no appreciable broadening due to the Doppler or the Stark effect, and are narrow enough to permit accurate measurement of the hyperfine separations.

The final spectrograms were obtained with a new 21 ft. concave grating spectrograph of the Eagle type. An outstanding feature of the instrument is the design of the plate holder. It has a length of 13 ft. and for an angle of incidence equal to 31.5° photographs the spectral region $n\lambda = 12{,}000$Å to $n\lambda = 21{,}000$Å in one exposure. Thus all lines with wave-lengths of 7000Å or less are recorded in at least two orders, provided the proper plates are used. The spectrograph is enclosed in a double walled room. By means of a thermostatic control the temperature of the room can be maintained so that the fluctuations at the grating are less than 0.01° C. The grating, recently procured from Professor Gale of Chicago, has a radius of curvature of 21.03 ft. and a total of 80,000 lines ruled 600 to the millimetre. This grating gives on one side of the normal a strong, well defined spectrum in the $n\lambda$ region quoted above. The maximum intensity is at $n\lambda = 17{,}000$Å and falls off gradually towards larger and smaller $n\lambda$ values. From the data given above the dispersion can be easily calculated for any value of $n\lambda$. For example, at $n\lambda = 20{,}000$ the dispersion in the fifth order is 0.383Å per millimetre.

A number of exposures ranging from one-half to three hours were taken on Wellington Anti-screen and Ilford Special Rapid Panchromatic plates.

Results and Interpretation

The measured structures are tabulated in Table I. The wave-lengths of the lines are given in the first column, their classifications (2, 8) in the second, and the hyperfine separations in the third. For each hyperfine

TABLE I

HYPERFINE STRUCTURE SEPARATIONS

Wave-length	Classification	Hyperfine structure separations in cm^{-1} $\nu \longrightarrow$							
3735Å	$5s6s\ ^3S_1 - 5s6p\ ^3P_0^o$	(10) 0.00 Sb_{121}	(8) 0.496 Sb_{123}	(6) 2.45 Sb_{123}	(8) 2.82 Sb_{121}	(4) 3.97 Sb_{123}	(4) 4.81 Sb_{121}		
3687Å	$5s6s\ ^3S_1 - 5s6p\ ^3P_1^o$	(5) 0.00	(3) 0.75	(4) 1.23	(4) 1.49	(10) 2.21	(3*d*) 3.14	(6) 4.21	(7*b*) 4.90
3426Å	$5s6s\ ^3S_1 - 5s6p\ ^3P_2^o$	(1) 0.00	(6*a*) 1.07	(4) 1.71	(10*ba*) 2.66	(2) 3.25	(9*ba*) 3.82	(1) 4.38	(4) 4.78
3288Å	$5s6s\ ^3S_1 - 5s6p\ ^1P_1^o$	(8) 0.00	(10) 0.63	(2) 1.22	(10) 2.72	(2) 3.55	(2) 4.14	(1) 4.69	(3) 5.60

NOTE:—*d—broad and diffuse; b—broad but well defined; a—slightly asymmetrical.*

pattern the positions of the components are expressed relative to the red component which is assigned the value 0.00. The intensities, given in brackets, were estimated visually relative to the strongest component which is arbitrarily assigned the value 10.

For the interpretation of the hyperfine patterns it is necessary to consider the contribution of each isotope. According to Aston (1) antimony has two isotopes, Sb_{121} and Sb_{123}, with a relative abundance $Sb_{121} : Sb_{123} = 100 : 80$. When this is taken into account the structures in Table I can be readily interpreted.

3735Å (5s6s $^3S_1$—5s6p $^3P^\circ_o$)

Theoretically the maximum number of hyperfine components for this multiplet transition is three per isotope. As six components have been observed the line must be completely resolved. The triplet structure for each isotope is characteristic of the $^3S_1$ term, since the $^3P^\circ_o$ is not split. The splitting of $^3S_1$ into three hyperfine levels shows immediately that the I of each isotope is $\geqslant 1$. The isotopic origins assigned in Table I to the six components are the only allotment consistent with Aston's abundance ratio and the interval rule which requires for $I \geqslant 1$ that the ratio of the intervals between the three components of each isotope be rational and $\leqslant 2$. For this allotment the hyperfine intervals of $^3S_1$ of Sb_{121} are normal and equal to 2.82 and 1.99 cm^{-1}, and those of $^3S_1$ of Sb_{123} are also normal and equal to 1.95 and 1.52 cm^{-1}. An application of the interval rule to these separations gives

$$Sb_{121} : \frac{I+1}{I} = \frac{2.82}{1.99} = 1.41$$

$$Sb_{123} : \frac{I+1}{I} = \frac{1.95}{1.52} = 1.28$$

These ratios definitely fix the I-values of Sb_{121} and Sb_{123} as $\frac{5}{2}$ and $\frac{7}{2}$ respectively.

The above interpretation is represented graphically in Fig. 1. The "calculated" pattern was derived by means of the interval rule from the total separations 4.81 and 3.47 cm^{-1} of the $^3S_1$ of Sb_{121} and Sb_{123} respectively, on the assumption that the centres of gravity of the patterns of both isotopes coincide. The coincidence of the observed and the calculated positions of the two central components confirms the interpretation. The observed intensities are consistent with those calculated by Hill's formulas (11).

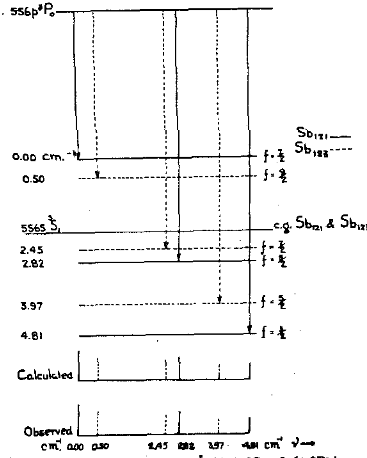

FIG. 1. *Structure of 3735Å (5s6s $^3S_1$—5s6p $^3P^\circ_o$).*

3687Å $(5s6s\ ^3S_1 - 5s6p\ ^3P_1^o)$

The structure of this transition is only partly resolved. For the I-values $\frac{5}{2}$ (Sb_{121}) and $\frac{7}{2}$ (Sb_{123}) it should have 14 components, 2 of which are expected to be weak (11). Eight components, of which one is diffuse, have been observed. The structure has been interpreted by the graphical method of Fisher and Goudsmit (7). The appropriate graph for a $J=1$ to $J=1$ transition was constructed using the I-value and the total $^3S_1$ hyperfine interval for each isotope obtained from 3735Å. The graphs for the two isotopes were superimposed with their c.g.'s coinciding to form a composite graph. The observed pattern fits this graph well for an interval factor ratio $A(^3P_1^o)/A(^3S_1) = 0.800$. The best fit indicates that the c.g. of Sb_{123} is 0.02 cm^{-1} to the red of c.g. (Sb_{121}), but this shift is not significant as it is of the same order as the uncertainty in the measured separations between components. The complete interpretation is represented graphically in Fig. 2. The positions of the components in the "calculated" pattern are those given by the composite graph for $A(^3P_1^o)/A(^3S_1) = 0.800$. The theoretical intensities (11) of the components are represented by the heights of the lines. The agreement between the calculated and the observed structure is good.

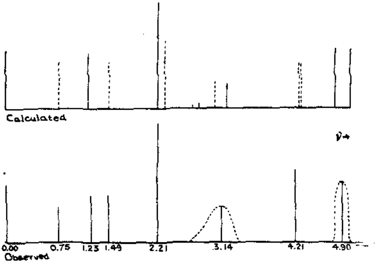

FIG. 2. *Structure of 3687Å* $(5s6s\ ^3S_1 - 5s6p\ ^3P_1^o)$.

3425Å $(5s6s\ ^3S_1 - 5s6p\ ^3P_2^o)$

Eight distinct components have been observed for this transition. The pattern was interpreted in a manner similar to that described for 3687Å. A satisfactory interpretation is obtained only when c.g. (Sb_{121}) is made to coincide with c.g. (Sb_{123}). The observed pattern fits the composite graph at $A(^3P_2^o)/A(^3S_1) = 0.420$. The observed and calculated patterns are compared in Fig. 3. The agreement is satisfactory.

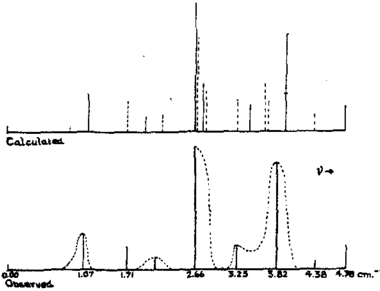

FIG. 3. *Structure of 3426Å* $(5s6s\ ^3S_1 - 5s6p\ ^3P_2^o)$.

3288Å $(5s6s\ ^3S_1-5s6p\ ^1P_1^o)$

Eight components have been observed for this transition. Although the pattern is comparatively weak, the outermost components appear with moderate intensity; hence the total hyperfine separation can be measured quite accurately. As this splitting is considerably greater than that of the $^3S_1$ (Sb_{121}) alone, the line pattern must be fitted to that portion of the graph corresponding to a negative value of $A(^1P_1^o)$. The graphical analysis shows that the c.g.'s coincide and that $A(^1P_1^o)/A(^3S_1)=-0.281$. The calculated and observed patterns are presented in Fig. 4.

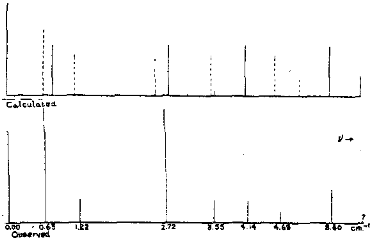

FIG. 4. *Structure of 3288Å* $(5s6s\ ^3S_1-5s6p\ ^1P_1^o)$.

The data obtained from the preceding analysis are summarized in Table II. No isotope shifts greater than the uncertainty in the writers' measurements (0.02 cm^{-1}) were observed. This means that the c.g.'s of the hyperfine levels of Sb_{121} and Sb_{123} coincide or have the same shift for all five terms in Table II.

TABLE II

TOTAL SEPARATIONS AND INTERVAL FACTORS

Term	Total Hfs. separation, cm^{-1}		Interval factor, A. cm^{-1}	
	Sb_{121}	Sb_{123}	Sb_{121}	Sb_{123}
$5s6s\ ^3S_1$	4.80	3.47	0.800	0.434
$5s6p\ ^3P_0^o$	Unsplit	—	—	—
$^3P_1^o$	3.84	2.78	0.640	0.347
$^3P_2^o$	4.03	2.91	0.336	0.182
$^1P_1^o$	−1.35	−0.98	−0.225	−0.122

Theoretical Discussion

g(I) Factors and Magnetic Moments

In the following the interval factors listed in Table II are compared with theory, and the interaction constants of the valence electrons are evaluated. The calculations are carried out only for Sb_{121}. Since the ratio of the interval factors of the two isotopes is constant $(A,\ Sb_{121})/(A,\ Sb_{123})=1.84$, for all terms, the interaction constants for Sb_{123} can be obtained directly from those for Sb_{121} by dividing the latter by 1.84.

5s6s—The expression for the interval factor of $^3S_1$ is

$$A(^3S_1)=\tfrac{1}{2}(a_{5s}+a_{6s})=0.800\ \mathrm{cm}^{-1}\ , \tag{1}$$

where a_{5s} and a_{6s} are the interaction constants of the two valence electrons. Since this is the only equation involving the two constants, they can not be evaluated directly from the interval factor. However, as a_{6s} is much smaller

than a_{5s} its contribution can be estimated with sufficient accuracy as follows. According to theory (21, p. 209, ref. 1; and p. 210) the expressions for a_{5s} and a_{6s} differ only in the factor

$$Z_i . Z_o^2 / n_{eff}^3 . \tag{2}$$

Z_i is the same for both electrons. For the 5*s* electron, $Z_o = 5$, $n_{eff} = 2.47$ as determined from the term value of 5*s* ^2S of Sb V. For the 6*s* electron, $Z_o = 4$, $n_{eff} = 3.50$ obtained by adding about one unit to the n_{eff} of 5*s*. The substitution of these values in Relation (2) gives

$$a_{5s}/a_{6s} = 4.45. \tag{3}$$

The values of the interaction constants obtained by substituting this ratio in Equation (1) are a_{5s} (Sb_{121}) = 1.31 cm^{-1}, a_{6s} (Sb_{121}) = 0.29 cm^{-1}.

5s6p—The coupling in this configuration tends towards (*jj*). For (*jj*) coupling the interval factor expressions are (9)

$$\begin{aligned} 5s6p_{\frac{1}{2}},\ J = 1 &: A(^3P_1^o) = \tfrac{1}{2}a_{5s} + \tfrac{1}{2}a_{6p}(\tfrac{1}{2}) \\ 5s6p_{1\frac{1}{2}},\ J = 2 &: A(^3P_2^o) = \tfrac{1}{4}a_{5s} + \tfrac{3}{4}a_{6p}(1\tfrac{1}{2}) \\ 5s6p_{1\frac{1}{2}},\ J = 1 &: A(^1P_1^o) = -\tfrac{1}{4}a_{5s} + \tfrac{5}{4}.a_{6p}(1\tfrac{1}{2}) \end{aligned} \tag{4}$$

where $a_{6p}(\frac{1}{2})$ and $a_{6p}(1\frac{1}{2})$ are the interaction constants of the 6*p* electron with $j = \frac{1}{2}$ and $j = 1\frac{1}{2}$ respectively, and a_{5s} is the interaction constant of the 5*s* electron. According to Goudsmit (10), and Fermi and Segrè (6)

$$a_{6p}(j) = \frac{R\alpha^2 Z_i Z_o^2}{n_{eff}^3\, l(l+1)\,(l+\frac{1}{2})} \cdot \frac{g(I)}{1838}\ \frac{l(l+1)}{j(j+1)} \cdot K(j, Z_i) = a_{6p} \cdot \frac{l(l+1)}{j(j+1)} \cdot K(j, Z_i), \tag{5}$$

where a_{6p} is an abbreviation for the first two terms, $K(j, Z_i)$ is the relativistic correction, and $j = \frac{1}{2}$, $1\frac{1}{2}$ for a *p* electron. For the 6*p* of Sb, $Z_i = (Z-4)$, and the corresponding relativistic corrections are $K(\frac{1}{2}, 47) = 1.26$ and $K(1\frac{1}{2}, 47) = 1.04$ (10). On substituting the values of $a_{6p}(j)$ obtained from Equation (5) in Equations (4) the latter reduce to

$$\begin{aligned} A(^3P_1^o) &= \tfrac{1}{2}a_{5s} + 1.68a_{6p} \\ A(^3P_2^o) &= \tfrac{1}{4}a_{5s} + 0.41a_{6p} \\ A(^1P_1^o) &= -\tfrac{1}{4}a_{5s} + 0.69a_{6p} \end{aligned} \tag{6}$$

Since the coupling is not strictly (*jj*), it is advisable to apply the above equations in the sum form rather than individually. The sum relations are

$$\begin{aligned} A(^3P_1^o) + A(^1P_1^o) &= \tfrac{1}{4}a_{5s} + 2.37a_{6p} = 0.415\ \text{cm}^{-1} \\ A(^3P_2^o) &= \tfrac{1}{4}a_{5s} + 0.41a_{6p} = 0.336\ \text{cm}^{-1}. \end{aligned} \tag{7}$$

Whence $a_{5s} = 1.28$ cm^{-1}, and $a_{6p} = 0.040$ cm^{-1}. Racah's equations (22) for intermediate coupling are also applicable to the individual interval factors of 5*s*6*p*, since the distribution of the terms is consistent with Houston's equations (12). In this case the interaction constants can be evaluated from any two of the interval factors and can be substituted in the expression for the third to test the consistency of the solution. Racah's equations are found to have a consistent solution and give the same values of a_{5s} and a_{6p} as obtained above. It is to be noted that the values of a_{5s} obtained from 5*s*6*s* and 5*s*6*p* are in good agreement.

The g(I) factor can be evaluated independently from one of a_{5s}, a_{6s}, and from a_{6p}. For the s electrons g(I) is given by the formula (10)

$$g(I) = \frac{8}{3}\frac{a_{ns}\cdot n_{eff}^3\cdot}{R\alpha^2 Z_i Z_o^2}\frac{1838}{K(\frac{1}{2}, Z_i)}. \qquad (8)$$

For $5s$: $Z_i=51$, $Z_o=5$, $n_{eff}=2.47$, $K(\frac{1}{2}, 51)=1.31$, $a_{5s}=1.3$ cm^{-1}. These values substituted in Equation (8) give g(I) = 1.4 proton magnetons. An independent evaluation of g(I) cannot be obtained from both a_{5s} and a_{6s}. In view of Relation (2) both must give the same value of g(I). For the $6p$ electron the formula for g(I) is (10)

$$g(I) = \frac{a_{6p}\cdot Z_i(l+\frac{1}{2})\cdot\lambda(l, Z_i)}{\Delta\nu}\quad 1838 \qquad (9)$$

For $6p$ of Sb IV: $Z_i=(Z-4)=47$, $l=1$, $\lambda(1, 47)=1.04$, $a_{6p}=0.040$ cm^{-1}, $\Delta\nu=\Delta^3P^o=2416$ cm^{-1}. These values substituted in Equation (9) give g(I) = 2.2 p.m.

The value of g(I) deduced from a_{5s} is by far the more reliable. The value obtained from a_{6p} is of the same order of magnitude but merits little weight: since, first, the value of a_{6p} is very small; second, the relativistic corrections used in Equations (7) are strictly valid for extreme (*jj*) coupling only; third, any slight perturbation of $5s6p$ by other configurations would produce a large percentage error in a_{6p}, and finally there is still some uncertainty as to the degree of accuracy of Equation (9).

In the derivation of g(I) from a_{5s} the values of Z_o and n_{eff} of the $5s$ electron of Sb IV were taken equal to those of the $5s$ electron of Sb V. The validity of the calculation then involves the assumption that the interaction constant of the $5s$ electron is not appreciably altered by the presence of the $6s$ electron. In the analogous two-electron spectra, Tl II, Pb III, Bi IV (16, 17, 18†, 19) the interaction constant of the more firmly bound *s* electron increases slightly as the second electron is removed to ionization. Thus g(I) = 1.4 calculated from a_{5s} of the $5s6s$ configuration is a lower limit. In Tl II, Pb III, and Bi IV the a_{6s} calculated from $A(6s7s\,^3S)$ by neglecting a_{7s} is slightly larger than the a_{6s} obtained from $A(6s\,^2S)$ of the next stage of ionization; *i.e.*, the contribution of the $7s$ electron to $A(^3S)$ more than compensates for the decrease in $A(^3S)$ produced by the screening effect of $7s$ on $6s$. It appears, therefore, that in antimony an upper limit for g(I) is obtained by neglecting a_{6s} and taking $\frac{1}{2}a_{5s}=A(5s6s\,^3S_1)=0.800$. This gives $a_{5s}=1.6$ cm^{-1}. g(I) computed from this value by Equation (8) is 1.7 p.m. These considerations place the value of g(I) between 1.4 and 1.7 p.m. g(I) = 1.6 appears from personal judgment to be a fair estimate.

The writers' analysis leads to a g(I) value appreciably larger than the value 1.1 derived by Goudsmit (10) from Badami's measurements. It is presumed that Goudsmit obtained his value from the data* on the $5p6s$ configuration of Sb II, since an analysis of this data by the writers gives his value. One

† *Also unpublished data.*

* *A number of the Sb II lines examined by Badami appear on the writers' spectrograms. The writers' measurements of the structures of these lines are in good agreement with Badami's.*

expects large mutual perturbation between the $5p6s$ and $5s5p^3$ configurations of Sb II, since they are adjacent and of the same parity. An application of Houston's equations (12) to the $5p6s$ terms confirms this. As the hyperfine structures of the $5s5p^3$ terms are large, the interval factors of the terms of both configurations probably depart appreciably from those predicted assuming no perturbation (6). In view of this there is some uncertainty in the value of g(I) deduced from the hyperfine structures of the $5p6s$ terms of Sb II. The $5s6s$ and $5s6p$ configurations of Sb IV are comparatively free from the above criticism. $5s5d\ ^3D_1$ and $5p^2\ ^3P_1$ appear to be the only terms that might perturb $5s6s\ ^3S_1$. Since these two terms have smaller interval factors than $^3S_1$ and are comparatively remote, their perturbing effect on $A(^3S_1)$, as in the analogous spectra Tl II, Pb III, Bi IV (5, 16, 17, 18*, 19), must be small (6). The $5s6p$ configuration apparently is unperturbed since Houston's equations are well obeyed. $4d^9 5s^2 5p$, which has not been identified, appears to be the only configuration likely to perturb; but since it has no unpaired electron its perturbing effect on $5s6p$ would introduce no appreciable error in the value of a_{6s} derived from the $5s6p$ interval factors. The consistency of the values of a_{6s} deduced from $5s6s$ and $5s6p$ further supports this conclusion. In view of the above considerations the value of g(I) obtained from Sb IV must be given more weight than the value obtained from Sb II.

The writers' data on the nuclear moments of antimony are summarized here:

Sb_{121} : $I=\frac{5}{2}$; $g(I)=1.6$; magnetic moment $=I.g(I)=4.0$ p.m.;
Sb_{123} : $I=\frac{7}{2}$; $g(I)=0.9$; magnetic moment $=I.g(I)=3.1$ p.m.;
$\frac{g(I)_{121}}{g(I)_{123}}=1.84$

Landé's Theory of Nuclear Moments

Landé (14) has calculated the $g(I)$ factors for the Z odd, M odd nuclei on the assumption that the nuclear mechanical and magnetic moments arise from the spin and orbital motions of a single proton. Combining the spin vector, $s=\frac{1}{2}$, and the orbital vector, l, he obtains the general equation for the nuclear g-factor

$$g(I) = g_l \cdot \frac{l(l+1)+I(I+1)-s(s+1)}{2I(I+1)} + g_s \cdot \frac{s(s+1)+I(I+1)-l(l+1)}{2I(I+1)} \qquad (10)$$

For g_s (magnetic factor of proton spin) $=4$ and g_l (magnetic factor of orbital moment) $=1$, he obtains striking general agreement between the calculated and observed $g(I)$ factors. However as the experimental $g(I)$ values are only approximate, the theory cannot be critically tested, especially in regard to the value of g_s. The data on antimony afford a more critical test. Although the $g(I)$ factors of the antimony isotopes are still subject to the uncertainty inherent in the theoretical calculations, the ratio of the $g(I)$ factors is accurately known to be equal to $1.82 \pm 2\%$ from both the writers' measurements and Badami's. Substituting the appropriate I and $g_l=1$ in Equation (10), the $g(I)$ of each isotope is obtained as a function of the l of the isotope and g_s. Dividing the expression for $g(I)_{121}$ by that for $g(I)_{123}$, the ratio $g(I)_{121}/g(I)_{123}$ reduces to a function of l_{121}, l_{123} and g_s. When the expression for this ratio is

* *Also unpublished data.*

equated to the experimental value 1.80 and solved, the following equation for g_s is obtained:

$$l_{121}(l_{121}+1) - l_{123}(l_{123}+1) = 7\,\frac{1+g_s}{1-g_s} \tag{11}$$

There are four possible solutions of this equation corresponding to the four l values that satisfy the relations $l_{121} \pm \frac{1}{2} = \frac{5}{2}$ and $l_{123} \pm \frac{1}{2} = \frac{7}{2}$. The solutions are given in Table III.

TABLE III

SOLUTIONS OF EQUATION (11)

l_{123} \ l_{121}	2	3
3	$g_s = -13$	$g_s = -1$
4	$g_s = 3$	$g_s = 15$

Any one of the four g_s values will satisfy the $g(I)$ ratio criterion for the $g(I)$'s of antimony; but according to Landé's theory only $g_s = 3$ is compatible with the sense and magnitude of the magnetic moments of not only antimony but also of the other Z odd, M odd isotopes.

The accurate experimental criterion $g(I)_{121}/g(I)_{123} = 1.80$ excludes for antimony Landé's value $g_s = 4$, since this value predicts $g(I)_{121}/g(I)_{123} = 2.4$. $g_s = 3$ does accurately satisfy the g(I) ratio in antimony, and in addition gives for other Z odd, M odd isotopes slightly better general agreement between the calculated and the experimental $g(I)$ values than can be obtained for $g_s = 4$. In Table IV the $g(I)$ factors calculated for $g_s = 3$ and $g_s = 4$ are compared with the experimental values.

TABLE IV

CALCULATED AND EXPERIMENTAL $g(I)$ FACTORS

Nucl. (Z)	I (obs.)	$g(I)$ (obs.) Goudsmit (10)	$g(I)$ (obs.) Fermi (6)	g(I) (obs.) Authors	g(I) (theor.) $g_s=3$	g(I) (theor.) $g_s=4$	l
Li_7 (3)	$1\frac{1}{2}$	2.19	—	—	1.7	2.0	1
Na_{23} (11)	$1\frac{1}{2}$	—	1.3	—	1.7	2.0	1
Al_{27} (13)	$\frac{1}{2}$	4.2	—	—	3.0	4.0	0
Cu_{63} (29)	$1\frac{1}{2}$	1.7	1.6	—	1.7	2.0	1
Cu_{65} (29)	$1\frac{1}{2}$	1.7	1.6	—	1.7	2.0	1
Ga_{69} (31)	$1\frac{1}{2}$	1.3	1.4	—	1.7	2.0	1
Ga_{71} (31)	$1\frac{1}{2}$	1.7	1.8	—	1.7	2.0	1
As_{75} (33)	$1\frac{1}{2}$	—	—	1.1*	{1.7	2.0	1
					{0.6	0.4	2
Rb_{85} (37)	$2\frac{1}{2}$	0.56	0.54	—	0.71	0.57	3
Rb_{87} (37)	$1\frac{1}{2}$	1.87	*1.85 (13)*	—	1.7	2.0	1
In_{115} (49)	$4\frac{1}{2}$	1.2	1.2	—	1.2	1.3	4
Sb_{121} (51)	$2\frac{1}{2}$	—	—	1.6	1.4	1.6	2
Sb_{123} (51)	$3\frac{1}{2}$	—	—	0.90	0.78	0.67	4
Cs_{133} (55)	$3\frac{1}{2}$	—	0.75	—	0.78	0.67	4
Tl_{203} (81)	$\frac{1}{2}$	3.6	2.8	—	3.0	4.0	0
Tl_{205} (81)	$\frac{1}{2}$	3.6	2.8	-	3.0	4.0	0
Bi_{209} (83)	$4\frac{1}{2}$	0.89	0.79	—	0.82	0.73	5

* *Calculated from the data by M. F. Crawford and A. M. Crooker (5).*

The experimental $g(I)$ values for Na_{23}, Cu_{63}, Cu_{65}, Ga_{69}, Ga_{71}, As_{75}, In_{115}, Sb_{123}, Cs_{133}, Tl_{203}, Tl_{205}, Bi_{209}, are in better agreement with the $g(I)$ values calculated for $g_s = 3$ than with those calculated for $g_s = 4$. For Li_7, Al_{27}, Rb_{85}, Sb_{121}, better agreement is obtained for $g_s = 4$. Rb_{87} is intermediate. The more accurate criterion of the $g(I)$ ratio can be applied only to Cu, Ga, Rb, Sb, and Tl. In the cases of Cu and Tl, for each of which the isotopes have the same I and $g(I)$ values, it is not possible to differentiate between $g_s = 3$ and $g_s = 4$, since the ratios are satisfied for any value of g_s. Neither $g_s = 3$ nor $g_s = 4$ satisfy the experimental $g(I)$ ratio in Ga. From Kopfermann's data (13), the $g(I)$ ratio for Rb is 3.40. This is considerably higher than the value 2.33 obtained for $g_s = 3$, but it is in fair agreement with the value 3.50 given by $g_s = 4$. As pointed out before, the $g(I)$ ratio in antimony is satisfied by $g_s = 3$, but not by $g_s = 4$. Thus summarizing: $g_s = 4$ satisfies the $g(I)$ ratio in Rb but not in Sb; $g_s = 3$ satisfies the ratio in Sb but not in Rb; both fail in the case of Ga; the cases of Cu and Tl are indecisive. The general agreement between the observed and calculated $g(I)$ factors favors $g_s = 3$. However, it appears since no specific g_s value satisfies the critical $g(I)$ ratio criterion for all cases, that the theory is incomplete as indicated by Landé.

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THE THERMAL DEHYDRATION OR DECOMPOSITION OF CERTAIN MINERALS AND SALTS[1]

BY A. F. GILL[2]

Abstract

A description is given of a thermal decomposition apparatus in use for some years in the author's laboratory. This consists of an improved form of the familiar combination of calcining furnace and weighing balance, which allows continuous weight determinations to be made of samples undergoing thermal decomposition. The accuracy of the results is enhanced by the provision of a thermocouple suspended directly in the charge and supported by the balance, the necessary connecting leads being as fine as possible without adding significantly to the resistance of the pyrometer circuit.

Thermal decomposition data are given for a number of minerals and salts which undergo loss of water or other constituents, and concerning which practical data of a reasonable degree of accuracy have from time to time been desired. These include, magnesite, hydromagnesite, magnesitic dolomite, dolomite, serpentine, asbestos, calcium and magnesium hydroxides, gypsum, hydrous magnesium sulphate, hydrous aluminium chloride, pyrite and certain samples of coal of high "volatile" content.

Introduction

There are numerous minerals and chemicals whose utilization depends in large measure upon calcination or roasting operations. Common reactions of this type are the decomposition of carbonates and hydroxides, and the dehydration of compounds containing water of crystallization.

As the temperature involved becomes higher, the difficulty in making accurate measurements of thermal decomposition points becomes correspondingly greater. As a consequence accurate data are lacking in the literature for a great many materials which are in comparatively common use.

Some years ago, information was required in this laboratory on the thermal decomposition of natural calcium and magnesium carbonates, both unassociated and as minerals of the dolomite class. While the decomposition point of calcium carbonate was known to a reasonable degree of accuracy, there was considerable conflict in the literature as to its behavior when associated with magnesium carbonate. Part of this uncertainty was due, no doubt, to the fact that vapor pressure measurements have been largely used in laboratory investigations. This method is not always a very convenient one, especially when the decomposition being investigated takes place in several stages, at relatively high temperatures.

Apparatus and Methods

As the information desired was essentially of a practical nature, work was undertaken with the more convenient apparatus which involves decomposition measurements by the suspension of the sample, in a furnace, from one arm of a balance. Apparatus of this type is well known, and is in daily use in, for example, the paper industry. Its disadvantage lies in the difficulty of

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obtaining accurate temperature measurement, as a thermocouple in the body of the furnace will not indicate accurately the temperature of a decomposing sample separately suspended. It was found, however, that if a sufficiently large apparatus were used, a thermocouple could be embedded in the charge itself without the necessary lead wires affecting unduly the sensitiveness of the balance.

An apparatus constructed in this way gave very satisfactory information on the calcium and magnesium carbonates, and quickly proved its value in the commercial developments arising from the results. Since that time, it has been used on various occasions in the laboratory when different materials have been under investigation. As some of the results are of general interest, there are given below a more precise description of the apparatus and some of the decomposition results obtained.

The balance used had a capacity of three kilograms. It was set up on an insulated table, sufficiently high above an electric muffle furnace to avoid any undue heating. The furnace was a well known multiple-heat design of 5 k.w. capacity. A large quartz crucible carried the charge. It was suspended in a cradle of nickel-chromium wire, from which a single strand extended through the furnace cover. A platinum-rhodium thermocouple was attached to the supporting leads, with its hot junction as near as possible to the centre of the charge. Fine copper leads connected the cold end of the thermocouple with the potentiometer. Cold-end temperatures were measured with a thermometer in close proximity to the wires, but hung independently of the crucible support. Calibration of the pyrometric equipment showed no significant reduction in millivoltage with the length of leads used. The crucible was fitted with a nickel cover (in some cases stainless steel) and the furnace itself was insulated at the top and bottom, in order to give a temperature as uniform as possible. A "pilot" thermocouple of base metal was kept in the body of the furnace. With this apparatus the accuracy of the weighings was well within 0.1 gm. With samples of 200 gm. or more, this gave a maximum error to this factor of less than 0.05%. During rapid decomposition, weighing was more difficult; errors were, however, of little practical significance, owing to the fact that at such times, temperatures tended to remain constant.

In early experiments, it was customary to raise the outer temperature at a uniform rate of about two degrees per minute. For materials of low thermal conductivity it was later found desirable merely to maintain a temperature difference of about 50° C. between the thermocouples.

Moderately coarse particles were found to give better results in the tests, and samples, when possible, were sized to minus 4 and plus 40 mesh. During steady decomposition there was little opportunity for air to enter the crucible containing the sample, although this would tend to happen after active evolution had ceased.

Under ideal conditions, such an apparatus would give a constant temperature for the whole period of decomposition. Actually, however, there is bound to be a rounding of the ends of the otherwise sharp curve, owing to

the fact that at the beginning of the decomposition there is lacking a full atmosphere of gaseous products, and more particularly that the outside of the charge is at a higher temperature than the central portion. While this detracts from the quantitative value of the results, their practical utility is not greatly affected.

Experimental Results

Natural Magnesium and Calcium Carbonates

Fig. 1 shows the decomposition of "Washington" magnesite, "Grenville" magnesitic dolomite and "Portage du Fort" dolomite. It was drawn from the original test data from which was developed a process of selective calcination of the magnesitic dolomite to effect decomposition of the magnesium carbonate in the presence of the calcium carbonate.

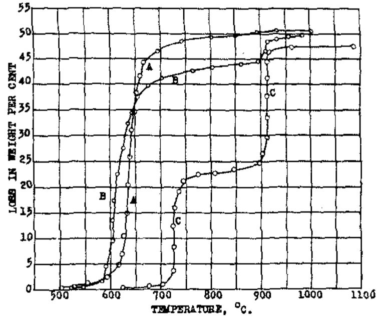

FIG 1. *A, magnesite; B, magnesitic dolomite; C, dolomite.*

Curve *A* shows the most constant value for the decomposition point of magnesium carbonate. The temperature of 640–650° C. may be taken as that of maximum decomposition. This value is of interest chiefly in confirming Curve *B*, which shows this point much less accurately.

This magnesitic dolomite is known, from mineralogical data, to be a mixture of magnesium carbonate and true dolomite ($MgCO_3 . CaCO_3$). Consequently, after the determination recorded in Curve *C* several attempts were made to obtain definite evidence of a similar decomposition point for the magnesium carbonate component of dolomite in this material. The failure to do this, together with the lack of definition of the normal magnesium carbonate decomposition, is ascribed in part to the shortcomings of the method as mentioned above and in part to the presence of some serpentine, the evolution of moisture from which would reduce the partial pressure of the carbon dioxide. It is evident, however, that the decomposition of normal magnesium carbonate is complete at about 650° C.

Curve *C* is of considerable interest in illustrating the behavior of the two carbon dioxide molecules in dolomite. It is seen that the magnesium carbonate component is stable to approximately 725° C., some 75° C. higher than the decomposition point of free magnesium carbonate. The calcium carbonate component, on the other hand, decomposes at the same temperature as free calcium carbonate, approximately 187° higher.

TABLE I

ANALYSES OF INORGANIC SAMPLES

Specimen	Magnesitic dolomite	Magnesite	Dolomite	Serpentine	Asbestos			Gypsum	Magnesium hydroxide	Calcium hydroxide	Magnesium sulphate	Pyrite	Hydromagnesite
					Canadian spinning stock	Arizona crude	Russian crude						
SiO_2	1.4	2.4	0.4	36.4	40.5	41.1	40.7	0.6	—	—	—	4.8	4.6
Al_2O_3	1.3	1.2	0.4	1.5	3.2	4.1	4.6	0.3	—	—	—		
Fe_2O_3					0.6	0.8	1.3					(Fe) 46.5	0.7
FeO	—	—	—	—	1.8	0.2	2.3	—	—	—	—	—	—
CaO	8.4	1.3	30.9	4.3	trace	0.5	trace	32.9	1.7	74.8	—	1.8	1.1
MgO	39.4	44.6	21.5	38.6	39.5	38.8	36.9	0.0	67.2	—	16.3	0.9	40.4
CO_2	49.5	50.5	46.8	5.7	0.2	0.6	0.2	2.6	2.0	2.1	0.2	—	36.5
H_2O comb.	—	—	—	12.0	14.0	14.9	14.3	19.7	29.2	23.2	50.8	—	15.8
H_2O free	—	—	—	1.5	—	—	—	—	—	—	—	—	—
SO_3	—	—	—	—	—	—	—	43.9	—	—	32.4	—	0.15
S	—	—	—	—	—	—	—	—	—	—	—	43.3	—

From the chemical analyses, which are given in Table I, the approximate combinations of carbon dioxide shown in Table II may be deduced, assuming all calcium oxide to exist in carbonates.

TABLE II

EMPIRICAL COMPOSITION OF CARBONATES

—	*A*	*B*	*C*
CO_2 as $CaCO_3$, %	1.0	6.6	24.3
CO_2 as $MgCO_3$, %	49.5	42.9	22.5

It will be noted that *A* and *C* do not give results concordant with the MgO as determined by analysis. More precise calculations are impossible, however, owing to the uncertain condition of the silica and other impurities.

British Columbia Hydromagnesite

Work was done some years ago on this material. The chemical analysis indicates roughly the empirical formula $5MgO. 4CO_2. 4H_2O$. Because it had been noted that the samples under investigation were alkaline to phenolphthalein it was thought possible that the material might be soluble in carbon dioxide solution without preliminary calcination. It was found, however, that while approximately 50% (by weight) dissolved quite readily, the remainder was attacked very slowly.

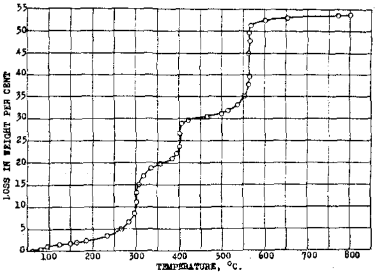

FIG. 2. *Hydromagnesite.*

The decomposition curve (Fig. 2) indicates positively two stages of evolution of carbon dioxide, *i.e.*, the higher one extends from 29% loss to 52.3%; the lower one presumably involves 13.2% carbon dioxide, which, however, in this curve cannot definitely be distinguished from water. Such a mechanism of decomposition would not agree with the empirical formula suggested above. It does, however, tend to substantiate the conclusion that this particular sample of hydromagnesite consisted of approximately 52% of a basic carbonate of the formula $3MgCO_3. 2Mg(OH)_2. 6H_2O$ mechanically mixed with an equal weight of normal carbonate, together with siliceous and other impurities. This matter was not considered sufficiently important for further investigation although light might be thrown upon it by microscopic studies. It might be observed that the accepted vapor pressure data for magnesium carbonate correspond with a decomposition temperature of approximately 550° C. The fact that the magnesium carbonate component of hydromagnesite decomposes at a temperature much closer to 550° C. than do the rock samples may be ascribed to the amorphous nature of the former which occurs naturally in a finely divided form.

Serpentine

Fig. 3 shows the thermal decomposition of a specimen of serpentine rock. The type of serpentine used in this determination occurs as an impurity in the magnesitic dolomite reported above. It will be noted that evolution of combined water occurs at a temperature substantially the same as that of the carbon dioxide of magnesite. This result was of interest in accounting for the fact that in the selective calcination of magnesitic dolomite in a commercial kiln the calcines always contained upwards of 0.5% water even when particular care was taken to eliminate air slaking during and after sampling. The evidence is that this represents residual water from undecomposed serpentine. This particular sample of serpentine was not very pure, as is shown in Table I. On the basis of the silica content it would contain 82.6% pure $3MgO.2SiO_2.2H_2O$, the balance being largely dolomite. Actually, however, there is some 1.3% more combined water than corresponds to this formula. Any carbon

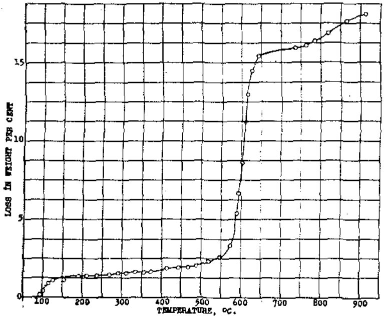

FIG. 3. *Serpentine.*

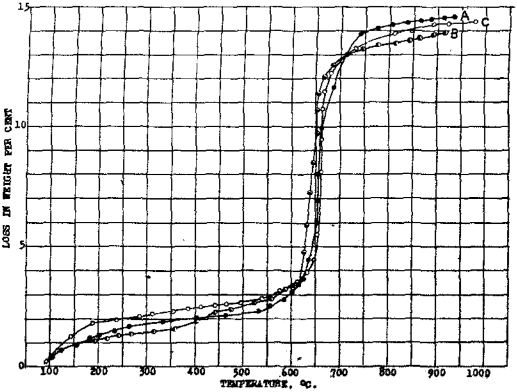

FIG. 4. ***Asbestos.** A, Arizona crude; B, Canadian spinning stock; C, Russian crude.*

dioxide existing in magnesium carbonate would be indistinguishable from the water of the serpentine in the decomposition curve.

Asbestos

In Fig. 4 decomposition curves are given for three different asbestos samples. As might have been expected, they are in rough agreement with the curve for serpentine rock. The dehydration of asbestos fibres is accompanied by loss of pliability and it thus destroys one of the most desired characteristics of the material. It is of interest to note that the Canadian crysotile material and Arizona and Russian crudes all undergo decomposition at substantially the same temperature. No explanation is offered for the apparent secondary decomposition of the Canadian material at approximately 400° C. The same break in the curve at this temperature was obtained in tests of three different samples of this material, indicating that it was not due to experimental inaccuracy. Two samples of African asbestos were also tested. The results, however, were unsatisfactory owing to its relatively small water content, coupled with the fact that weight losses were masked by oxidation of ferrous iron which is present in relatively large amount.

The analyses, which are reported in Table I, show the materials to be all of the serpentine type although, like the serpentine, they tend to be higher in water content than the pure compounds.

Calcium and Magnesium Hydroxides

The decomposition temperatures are shown in Fig. 5. While somewhat indefinite, they are useful approximations. The indicated point for magnesium hydroxide is approximately 375°–380° C., and that for calcium hydroxide 520–525° C. These materials were prepared in the laboratory by calcining and rehydrating chemically pure hydroxides. The water contents of pure calcium and magnesium hydroxides are respectively 24.3 and 30.9%.

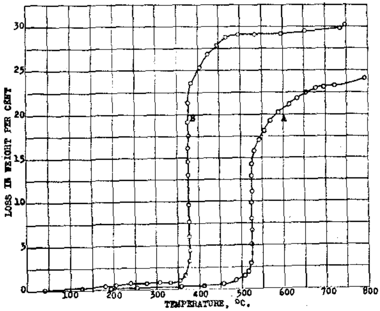

Fig. 5. *Calcium and magnesium hydroxides. A, calcium hydroxide; B, magnesium hydroxide.*

Gypsum

The material reported here was of the massive variety, it having previously been found that the points obtained with the crystalline form (selenite) were not as sharply defined. The two stages of decomposition are shown quite distinctly in Fig. 6, although the proportion in each stage is not definite

owing to the temperature lag. In commercial calcination of gypsum, the first "boil", or loss of 75% of the water, is considered to occur at 120–130° C. and the second at 180–195° C. These determinations show the more definite points of 118° and 192–193° C. respectively for the two "boils". Numerous investigations have been made of the decomposition of gypsum. Stumper (1) obtained values varying from 125–127° to 140° C. for the lower temperature and 190–192° C. for the higher.

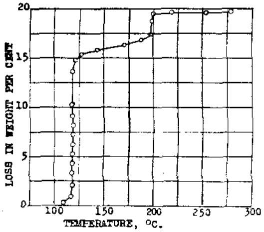

FIG. 6. *Gypsum.*

Magnesium Sulphate

The laboratory sample used was the heptahydrate of reagent grade. The curve (Fig. 7) is of interest in showing the two stages of dehydration. The temperature of 110° C. corresponds with the loss of six molecules of water, the remaining molecule coming off at approximately 350° C.

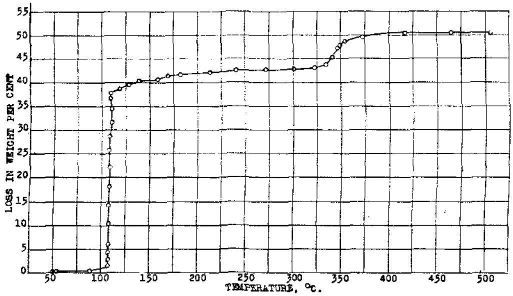

FIG. 7. *Hydrous magnesium sulphate.*

This information was used in a study of the transfer of water from crystalline magnesium sulphate to plastic magnesia, when the former was added as a neutralizing agent for free lime.

Aluminium Chloride, Hydrous

The thermal decomposition of hydrous aluminium chloride to yield a residue of aluminium oxide is well known. In connection with a study of certain proposed methods of dehydration, it became desirable to have accurate data on the temperature at which this decomposition actually takes place. This curve (Fig. 8) shows the decomposition point very satisfactorily. It is of interest also to note that the temperature, 182° C., coincides with the accepted boiling point for anhydrous aluminium chloride. The sample used

was of reagent grade. The break in the curve shortly above 100° C. is evidently due to loss of hygroscopic water.

Pyrite

Some attention is being given in this laboratory to the recovery of elemental sulphur from Canadian pyrite ores and mill tailings. This has involved consideration of the thermal decomposition of pyrite which takes place approximately according to the equation, $FeS_2 = FeS + S$. This determination was made in order to give more accurate information on the temperature of this reaction.

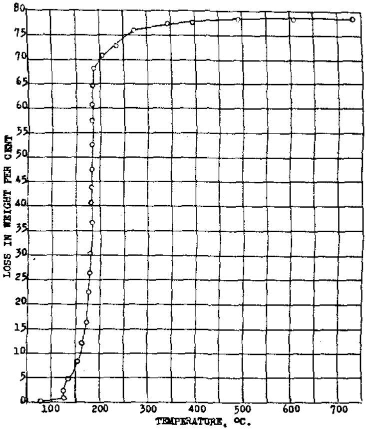

FIG. 8. *Hydrous aluminium chloride.*

In the curve (Fig. 9), the loss in the vicinity of 100° C. indicates moisture. Unfortunately no determination of moisture was made on the sample actually used. The break in the curve at approximately 8% loss indicates two stages of decomposition but no accurate indication of the intermediate product can be obtained from these data. At any rate, it is evident that the major decomposition is complete at 680–685° C.

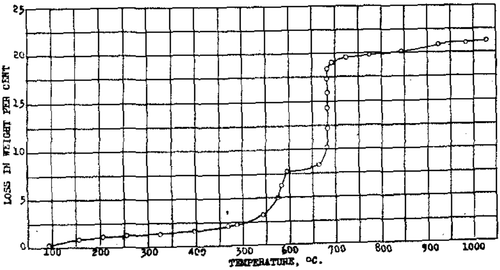

FIG. 9. *Pyrite.*

Coal

As a matter of interest, results are shown in Fig. 10 of tests on three samples

of western Canadian coals. The curves indicate the characteristics of the various materials to some extent. The data in general are an indication that this method is useful in presenting a quick picture of the moisture content together with the range over which the volatile organic matter is distilled. It is to be noted that the reported proximate analyses do not agree with the test results, owing largely to differences in the water content. Calculations on the dry basis show fair agreement when the arbitrary nature of the determination of volatile matter is considered.

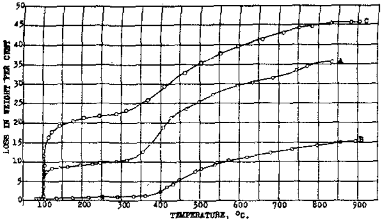

FIG. 10. *Coal samples.*

TABLE III

ANALYSES OF COAL SAMPLES

—	A	B	C	—	A	B	C
Moisture, %	10.3	1.4	18.7	Volatile matter, %	33.0	17.5	31.0
Ash, %	5.7	3.7	4.8	Fixed carbon, %	51.0	77.4	45.5

TABLE IV

INDICATED DECOMPOSITION POINTS

Substance	Decomposition point, ° C.	Substance	Decomposition point, ° C.
Magnesite	650 (max.)	Asbestos	625–660
Hydromagnesite		Calcium hydroxide	520–525
(a) Water (?)	300 (max.)	Magnesium hydroxide	375–380
(b) Carbon dioxide	400 (max.)	Gypsum	118 and 192–193
(c) Carbon dioxide	565 (max.)	Magnesium sulphate (hydrous)	100–110 and 330–350
Dolomite		Aluminium chloride (hydrous)	182
(a) CO_2 from $MgCO_3$	725	Pyrite	680–685 (max.)
(b) CO_2 from $CaCO_3$	912		
Serpentine	600–625		

Acknowledgment

Acknowledgment is made to Mr. C. W. Davis of these laboratories, who made a number of the analyses and performed some of the tests.

Reference

1. STUMPER, R. Compt. rend. 184 : 970-972. 1927.

HYSTERESIS IN SILICA GEL SORPTION SYSTEMS[1]

By L. M. Pidgeon[2]

Abstract

The hysteresis which normally appears in the isotherms of the silica gel-water system has been attributed by Patrick to the presence of permanent gases in the system. Only one case has been found in which a reversible isotherm has been recorded in the silica gel-water system. For alcohol and benzene only one case of hysteresis has been reported. These results seem to be independent of the presence or absence of air or other gases.

The sorption of water, benzene and ethyl alcohol has been examined using the sorption balance. A hysteresis loop appears for water only. This hysteresis may not be eliminated by special methods of evacuation and must be considered as a real effect. The isotherms of alcohol and benzene, on the other hand, are completely reversible. It has been shown that the dimensions of the hysteresis occurring in the water system may be affected by the manner of addition of vapor to the apparatus. Only when the vapor pressures remain reasonably constant during sorption are the dimensions of the effect evident. If very large pressure changes take place the hysteresis may disappear.

A comparison of the isotherms for water showing hysteresis, and those of the sulphur dioxide system (upon which the original suggestion as to the cause of hysteresis was based) show that there is not necessarily any relation between the two.

Introduction

The early workers on the sorption of water by silicic acid gels invariably reported isotherms in which the equilibrium values obtained by sorption from lower pressures were not as high as those obtained by loss of water from a more saturated condition. A hysteresis loop of characteristic shape was thereby produced which has been the subject of much subsequent discussion and for which a satisfactory explanation is still lacking.

More recently Patrick (9) and his collaborators, who have been responsible for much of the later work on sorption by silica gel, suggested that this hysteresis has no real existence and is simply due to the incomplete removal of permanent gases from the sorbent. This explanation of the phenomenon, based on experiments carried out on the sorption of sulphur dioxide by silica gel was extended to embrace all cases of sorption hysteresis (10, p. 1278), and as such has been accepted by a number of workers in this field (7, p. 184).

On the other hand it has been questioned, in the case of water at least, and considerable evidence produced to show that the hysteresis is a real effect and not connected with experimental error.

In this paper it is the author's intention to discuss the evidence for and against the conclusion that hysteresis is due to traces of permanent gases, and to present the results of experiments which have been carried out on the sorption by silica gel of the vapors of water, ethyl alcohol, and benzene, with special reference to the occurrence of hysteresis in the isotherms of these systems.

[1] *Manuscript received March 29, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

[2] *Chemist, National Research Laboratories, Ottawa.*

Discussion

van Bemmelen (17), and Anderson (1) were among the first to examine the sorption of water vapor by silica gel. They employed the static method of measurement using sulphuric acid solutions of known strength to produce a constant vapor pressure in their apparatus. van Bemmelen's experiments were carried out in the presence of air, while Anderson used a vacuum method, although the conditions were such as to prevent very complete removal of inert gases. In all cases the characteristic hysteresis loop appeared in their isotherms.

Subsequently Patrick (12) using the dynamic method in air produced a similar hysteresis loop, although alcohol and benzene under the same conditions yielded reversible isotherms.

However Patrick (9) had already carried out the experiments on the silica gel–sulphur dioxide system, upon the results of which were based his conclusions regarding the cause of the hysteresis. In this work it was clearly shown that if small amounts of air were introduced into a thoroughly evacuated static sorption system, a hysteresis appeared which had not been present before and which could be removed by rigorous evacuation of the sample. This result seemed quite definite, and the suggestion was made that the explanation could be extended to include all cases of hysteresis. The application of such a suggestion to sorption by organic fibres was adversely criticized by Urquhart (16), who at the same time questioned its applicability to the case of silica gel itself, and turned his attention to this system. Using a static method he reported the familiar hysteresis loop for water and silica gel even after very careful evacuation technique. Whereupon Patrick (11) again examined the sorption of water, this time using a static method and taking special precautions to ensure removal of inert gas. (The samples were heated to 350° C. and evacuated a number of times and in the intervening periods were allowed to sorb water at 20° C. The isotherms which were obtained after this treatment were perfectly reversible and showed no trace of hysteresis. These results were regarded by the author as conclusive proof of his previous contention.

More recently, however, Lambert and Foster (6) examined the water-silica gel system and, in spite of the fact that they employed even more careful methods of evacuation than Patrick, and used a much more compact apparatus, a definite hysteresis loop made its appearance in their isotherms.

Similar confusion is to be found in the results of experiments on the sorption of the vapors of alcohol and benzene. Anderson (1) described a hysteresis loop in the isotherms of both of these systems which was similar in shape to that of water. On the other hand, Lambert and Clark (5) using the same elaborate evacuation technique as for the water system, found no hysteresis in either case. McBain (7, p. 188) reproduces the isotherms of both of these authors as examples of the differences which follow when evacuation is thorough. Referring to Patrick's experiment on the water

system in which the isotherms were reversible, he states that, "Lambert and Clark have performed a similar service for the isotherms of benzene and silica gel." Uncertainty again arises when it is remembered that Patrick (12) had already demonstrated that when the vapor is carried over the sorbent in a stream of air, water produces a hysteresis but alcohol and benzene do not, while as already mentioned, Lambert and Foster (6) applying the same technique they had used with benzene found a hysteresis in the case of water sorption.

It appears that the mass of evidence suggests that a real hysteresis loop appears in the isotherms of the water-silica gel system, while the isotherms of alcohol and benzene are perfectly reversible. Only in the static experiments of Patrick (11) have perfectly reversible isotherms appeared for water sorption and only one author (1) has found hysteresis in the case of benzene and ethyl alcohol. The appearance or disappearance of the hysteresis loop in these cases is apparently independent of the presence or absence of air or other gases.

Considerable importance has been attached to this experiment of Patrick's on the water-silica gel system by McBain (7, p. 185) who states, "It is most interesting to observe that the form of these isotherms for water in the absence of air and volatile impurities differs wholly from those previously obtained and resembles ordinary isotherms for charcoal. . . . To the author, it appears probable that if the drastic methods of evacuation discussed in Chap. IV were here available, the sorption isotherms would approach in form those developed for charcoal by means of the sorption balance, practically all the sorption taking place at extremely low pressures. The incompatibility of such behaviour with the hypothesis of sorption as capillary condensation has already been mentioned."

The establishment of the true position of the phenomenon of hysteresis is therefore a matter of some theoretical importance as its presence, if established, will present a fact which must be accounted for in any theoretical explanation of the mechanism of sorption.

Possible Causes of the Disappearance of Hysteresis in Certain Cases

The experiments of Patrick which showed no hysteresis are of sufficient importance to warrant detailed attention. It seems from the survey which has been made that in the case of water, the hysteresis may not be removed by careful evacuation, and the analogies which have been drawn with benzene and alcohol are incorrect. Some other cause for the disappearance of the loop must be found. The author believes that this may be discovered in the experimental method which was employed by Patrick in this particular research.

While no numerical values are given for the sorption results (11), some details of the apparatus appear. It is mentioned that 1200 cc. of gel was divided into 12 samples, each being enclosed in a suitable bulb and all connected through suitable mercury "cut-offs" to a pump, manometer and water reservoir. After evacuation, as previously described, the apparatus was filled

to various pressures with water vapor and a bulb sealed off at each measured point. The moisture content of the gel in each bulb was accurately determined by several methods. The points to which attention must be drawn are, first, that a very large amount of gel was present in the sorption system, and, second, that the alteration of vapor pressure was apparently achieved by direct addition or removal of water vapor. It is suggested that these two factors have been responsible for the disappearance of the hysteresis in these experiments.

It has been pointed out by Seborg and Stamm (15), and also by Campbell (2), that when the amount of vapor is controlled by admitting or withdrawing small amounts from a sorption system, an error is introduced when hysteresis is under examination. This is due to the fact that the direct addition of vapor to the system raises the pressure above the equilibrium value, so that the subsequent sorption takes place under a falling vapor pressure. Under these circumstances, while the system as a whole would move from an equilibrium at low vapor pressure to one at higher vapor pressure, in certain portions of the gel the process might be reversed. The more accessible parts of the sample might quickly come to equilibrium with the higher pressure of the newly admitted vapor, hence in the later stages of sorption these portions would approach equilibrium by *loss* of vapor, while the rest of the sample is gaining it. Hence the process of sorption and desorption would be taking place simultaneously. The reverse effects, of course, take place on a descending isothermal; as the vapor pressure surrounding the sample is lowered below the final equilibrium value and rises to it as equilibrium is reached.

It will be apparent that if these pressure changes are very marked there will be a possibility that, should a hysteresis exist, its presence might be obscured by the confusion of sorption and desorption, for it is conceivable that the amount of each process taking place simultaneously might exactly balance and a reversible point appear in the resulting isotherm.

The magnitude of this obscuring effect will obviously depend on pressure change which takes place during the establishment of equilibrium at any given pressure. This in turn depends to some extent on the size and nature of the gel particles, but more particularly on the ratio existing between the amount of vapor taken up by the sample between successive points and the amount of vapor in the free space of the sorption system. The greatest changes follow when a large amount of gel is enclosed in an apparatus of small free volume, while in an apparatus of large volume the fall in pressure brought about by removal of vapor by a small sample will not be large and the error from this source will be minimized. Very slow addition of vapor also reduces the error as the pressure in the system is not raised far above the new equilibrium point. Campbell and others have eliminated all uncertainty on this point by maintaining a constant vapor pressure in the system by the use of solutions of the appropriate strength.

If the experimental method of Patrick is examined in the light of this suggestion it will be found the conditions holding were particularly unfavor-

able. The method which was employed to determine the moisture content of the gel, while very accurate, necessitated the use of an unusually large amount of gel, so that the ratio of gel to apparatus was very great (1200 cc. of gel was originally present, which compares with 14 gm. in Lambert's apparatus and 2 gm. in the experiments to be described later) and the pressure changes might well be sufficient to obliterate the hysteresis.

This same criticism may be applied to the experimental methods which have been employed by a number of investigators, and in general applies to the "standard sorption method" (7, p. 12), but in no case under discussion were the conditions as unfavorable as in that referred to.

Further evidence in favor of this suggestion is found in the work of Lambert and Clark (5). These authors employed a method in which a pressure change of approximately 2 cm. of mercury existed between the vapor in the reservoir and that in the sorption system during admission of vapor. This pressure change was approximately equal to a change in p/p_s of 10%, and was not sufficient to hide the hysteresis, though its dimensions may be affected (see Figs. 4 and 7). While the characteristic loop appeared for water sorption, the authors observed that its dimensions could be altered by the manner of addition of vapor to the system. It was found that where the descending and ascending isothermals coincided, the manner of addition of the vapor produced no effect on the sorption values, but over the region where hysteresis appeared, interruptions in the admission of vapor produced points lying within the loop. "By these interruptions of smooth addition or withdrawal of benzene, it was possible to obtain equilibrium values within the hysteresis area . . . The ascending isothermal, obtained by smooth successive additions of benzene to the reaction system set an upper limit to the equilibrium pressures just as the corresponding "descending" isothermal set a limit to the equilibrium pressure."

(These observations were first made by Lambert and Clark (5) on the system ferric hydroxide–benzene which shows a similar hysteresis to that of water and silica gel. They were later repeated by Lambert and Foster (6) in the latter system.)

The interruptions referred to consisted in adding a known amount of vapor to the system, after which a portion was withdrawn. The sorption value obtained in this way was higher than that which would have appeared if the point had been reached by sorption in a regular way from a lower saturation point. It will at once be observed that this is actually what happens when a gel sorbs vapor in a closed system without special means to ensure a constant pressure. As the gel takes up the vapor the pressure in the system falls exactly as if a portion had been withdrawn by a pump or other agency as described by Lambert and Clark. Hence when hysteresis appears its true dimensions will be apparent only when the vapor pressure during the addition of vapor to the system does not rise above the equilibrium at the new point.

The remaining part of this paper describes experiments which have been carried out to test the validity of these suggestions. Experimental methods

have been adopted in which the amount of pressure change taking place during sorption may be varied, and the effect on the hysteresis of this variation studied.

Experimental

The McBain-Bakr sorption balance has been employed exclusively, as it is the most convenient method of measurement in which the determination of the amount of sorption is independent of the pressure of the vapor or the amount of liquid in the reservoir. The method of admission of vapor, and the pressure changes taking place during sorption may therefore be varied at will without affecting the sorption determination.

Apparatus

The general appearance of the apparatus is apparent from Fig. 1. The samples were placed in light buckets constructed of aluminium gauze and suspended from the spirals as indicated. The quartz springs were constructed as previously described (14). Extensions were measured with a cathetometer. The weight of the sample was determined with an accuracy of 0.1% except where otherwise stated. In order that various organic solvents could be examined, all stopcocks were eliminated and their place taken by mercury "cut-offs". The ground joints over the sample tubes were used satisfactorily in the dry condition with mercury seals. The system was evacuated with a high speed, two stage, Gaede diffusion pump backed by the customary oil-immersed mechanical pump. Pressures during evacuation were measured with a McLeod gauge, and the vapor pressure during sorption was measured on the manometer, readings being made with the cathetometer.

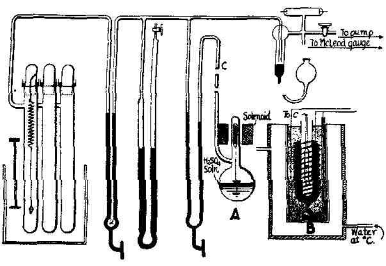

FIG. 1. *Sorption balance modified for production of constant pressures in the system.*

The general operation of the balance is obvious from the diagram. The special features having to do with the admission of vapor, pressure control, and the precautions taken to ensure the removal of permanent gases from the system will be described in subsequent sections, together with the results obtained with each method.

Materials

Commercial silica gel (−8 to +14 mesh) was employed in all experiments. The gel was obtained from two sources, the samples from each showing the same general results with small differences in the actual sorption values. The

gel was placed in the apparatus as received and in most cases was not heated prior to sorption. Pure benzene (Schuchardt) and absolute ethyl alcohol were employed in the sorption experiments on these substances.

Experimental Results

I. Sorption of Water Vapor

(a) Sorption During Constant Pressure

If the suggestions which have been set forth in the preceding section are correct, the true dimensions of the hysteresis should appear only when sorption takes place with a constant vapor pressure in the free space of the sorption system. This has been achieved in two ways:

1. Sulphuric Acid Solutions

The details of this method are shown in *A*, Fig. 1, and are in general similar to those described by Campbell (2). The constant vapor pressure of water was obtained by the use of sulphuric acid solutions of suitable concentrations immersed in the same thermostat as the samples (shown separately in diagram). A stirrer, operated by a solenoid, continually broke the liquid surface during the course of an experiment.

One litre of solution was used, so that with the average weight of sample which was employed, the change in relative humidity resulting from alteration in acid strength during sorption did not exceed 0.008%. This change in humidity is approximately equal to a temperature change of 0.01° C., which corresponded to the limit of accuracy of temperature control.

The samples were evacuated until no gas was given off and their weight determined by reading the extension of the spirals. They were then "washed" with water vapor, followed by evacuation, this process being repeated several times. The acid solution was then placed in communication with the samples and 24 hr. was allowed to elapse before taking the final reading, the solution being stirred throughout this time. The vapor pressure in the apparatus was then raised to a higher level by admission of water vapor from a separate reservoir, after which a descending point was obtained by again placing the samples in communication with the acid solution for 24 hr., after which the solution was diluted by the addition of freshly boiled distilled water and the above process repeated at a higher vapor pressure.

2. Thermostatic Control

The above method, while giving accurate control of the vapor pressure and having the sanction of previous investigators, suffered from two serious faults. The operations required to pass from one point to another on the isotherm were tedious, while the frequent opening of a part of the apparatus required by admission or withdrawal of sulphuric acid or water, rendered it difficult to ensure complete removal of permanent gases.

To obviate these difficulties the apparatus was modified as shown in *B*, Fig. 1. The control of vapor pressure was then effected by accurately main-

taining a known temperature around a water bulb placed in communication with the sorption system. A special thermostat previously described (14) was employed in this connection. Ice water was circulated through the jacket enabling constant temperatures as low as 4° C. to be maintained in the water bulb. The temperature fluctuations with this apparatus were barely detectable with a Beckmann thermometer and were unlikely to be greater than 0.005° C. With this arrangement the constancy with which the relative humidity within the apparatus may be maintained depends on the accuracy of temperature control of this thermostat and that surrounding the samples. The latter was equipped with a specially sensitive thermoregulator which gave regulation to better than 0.01° C., so that variations of relative vapor pressure were well below the accuracy of sorption determination.

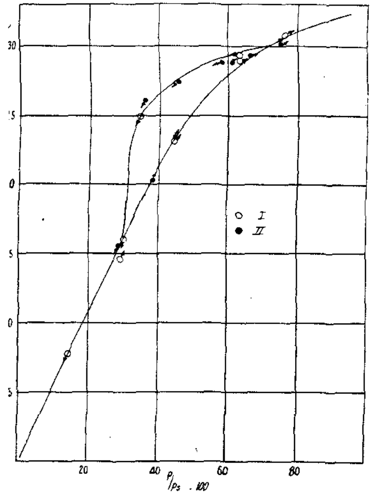

FIG. 2. *Sorption of water vapor by silica gel; constant pressure during sorption. T = 20° C.*

With this modification of the apparatus it was possible to remove dissolved gases from the water by freezing and evacuation in the ordinary manner, while the various vapor pressures required for the production of the complete isotherm were readily obtainable by adjustment of the temperature of the thermostat. The temperature of each thermostat was checked with standard thermometers and the ratio p/p_s obtained from tables.

Experimental Results

Results of experiments carried out with constant vapor pressures during sorption are given in Table I and appear in Fig. 2, where "x" is the weight of water sorbed and "m" the weight of the sample. The points are identified as ascending and descending according to the direction of the vapor pressure change which has taken place.

It is at once apparent that the characteristic hysteresis is present in both cases. This type of loop is identical in shape with that reported by the various authors referred to in the introduction. Various samples of gel showed the same effect, though the actual values might be somewhat different, as seen in Table I.

TABLE I

RESULTS OF EXPERIMENTS AT CONSTANT VAPOR PRESSURES

Apparatus A. Temp., 20° C.						Apparatus B. Temp., 20.38° C.					
	Silica gel			Silica gel			Silica gel			Silica gel	
$p/p_s \cdot 100$	No. 1	No. 2	$p/p_s \cdot 100$	No. 1	No. 2	$p/p_s \cdot 100$	No. 1	No. 2	$p/p_s \cdot 100$	No. 1	No. 2
	$x/m \cdot 100$			$x/m \cdot 100$			$x/m \cdot 100$			$x/m \cdot 100$	
Ascending points			*Descending points*			*Ascending points*			*Descending points*		
29.0	14.6	14.5	76.7	36.7	30.7	44.8	24.6	23.4	63.4	34.8	29.8
44.1	24.5	23.1	63.5	35.1	29.3	60.8	30.9	28.9	58.0	33.7	28.8
63.4	33.8	28.9	34.4	26.1	24.9	66.1	34.7	29.3	45.4	32.7	27.5
76.7	36.7	30.7	29.9	14.8	15.9	75.1	35.3	30.1	35.6	29.3	26.1
			14.2	7.9	7.8				28.6	14.3	15.5
									Ascending point		
									38.4	20.2	20.2

(b) Sorption After Thorough Evacuation

In view of the very thorough evacuation employed by Patrick and others, a criticism might be leveled at the above work on the grounds that the samples were not heated during evacuation, while temperatures up to 440° C. had been employed by various authors. In order to investigate this point a special form of the sorption balance was constructed as illustrated in Fig. 3. The apparatus was constructed of Pyrex glass and the dimensions were kept small so that all parts could be heated by a free flame during evacuation. The "cut-offs" were of small size and completely enclosed and the mercury was boiled during evacuation. The manometer was constructed according to the recommendations of Lambert and Clark (4). The boiled water was introduced into tube *B* and frozen twice with evacuation before being distilled into the tube *A* and sealed off at *C* as shown. The freezing and thawing during evacuation was then repeated. The pumping system was operated until a zero reading was obtained on the McLeod gauge, after which the apparatus was tested for gas tightness over a period of several days. A sample of silica gel was placed in a glass bucket and suspended from a specially sensitive spiral (1 part to 10,000) by a fine glass thread. The following operations (i and ii) were then repeated three times:

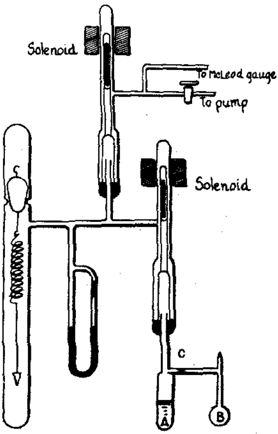

FIG. 3. *Sorption balance modified for careful evacuation of samples.*

(i) The gel was heated with a jacket furnace to 300° C. evacuated to the limit of the gauge. (ii) The gel was cooled to 20° C., and water vapor admitted; the vapor pressure rose to 4–10 mm. After this treatment the sorption points were taken in the ordinary manner, 24 hr. being allowed for the establishment of equilibrium in the case of the points falling within the hysteresis loops; longer times up to several days made no difference in the readings.

The vapor pressure during these experiments was not absolutely constant during the establishment of equilibrium, but the ratio of gel volume to free volume of apparatus (0.5 gm. of gel employed) was such that this change was very small and unlikely to affect any hysteresis which might be found. The results which appear in Table II and in Fig. 4, *B*, show the same loop as the previous isotherms. In fact the loop is even wider than those appearing where the evacuation methods had been less elaborate. The treatment has not affected the hysteresis, though the actual sorption values have been reduced by the heating. Actually, while the values are lower than those found previously the curves are affine except for a portion of the loop. It may be mentioned that no attempt was made to exclude mercury vapor from the apparatus, as it was apparently present in Patrick's experiment.

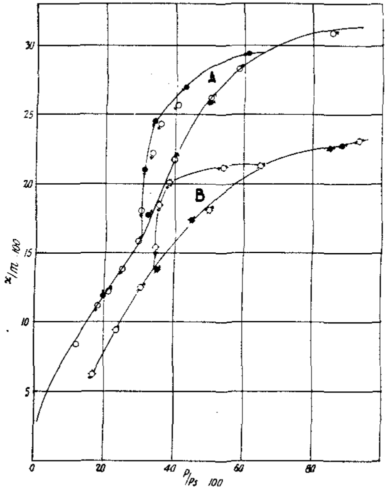

FIG. 4. *Sorption of water by silica gel. A, small pressure variations; B, sample rigorously evacuated.*

TABLE II

SORPTION OF WATER BY SILICA GEL AFTER THOROUGH EVACUATION (TEMP. 24.0°C.)

p/p_s . 100	x/m . 100	p/p_s . 100	x/m . 100	p/p_s . 100	x/m . 100	p/p_s . 100	x/m . 100
Ascending points		*Descending points*		*Descending points*		*Ascending points*	
42.6	15.1	54.2	21.2	34.2	15.4	23.5	9.3
50.0	18.1	38.3	20.1	30.6	12.5	35.5	13.8
64.8	21.3	35.3	18.5	23.8	9.4	44.8	17.4
93.2	23.1			16.2	6.2	85.0	22.6
						88.2	22.8
						Descending point	
						36.5	18.6

(c) Attempts to Remove the Hysteresis by Pressure Changes During Sorption

It appears from the foregoing that if a reasonably constant vapor pressure is maintained during the sorption of water by silica gel a characteristic hysteresis loop is formed by the curves of ascending and descending points. This loop may not be removed by special methods of evacuation as has been stated. This result is in accord with the findings of Urquhart, Lambert and coworkers, and the earlier workers on sorption of water by this substance. The suggestion has been made that the disappearance of the hysteresis in the one case in which it did not appear may have been due to the large pressure changes which took place during sorption. Experiments have therefore been carried out in which no precautions were taken to ensure the constancy of pressure during the attainment of equilibrium at any given point. The apparatus as illustrated in Fig. 1, *B*, was employed with the exception that the thermostat (*B*) was omitted and various amounts of water vapor were directly added to the apparatus after which the "cut-off" was closed, so that the vapor pressure would fall as equilibrium was reached. The results of these experiments appear in Table III and in Fig. 4, *A*.

TABLE III

RESULTS OBTAINED WITH VARIABLE VAPOR PRESSURE (SMALL PRESSURE CHANGES)

$p/p_s \cdot 100$	Silica gel No. 1	Silica gel No. 2	$p/p_s \cdot 100$	Silica gel No. 1	Silica gel No. 2	$p/p_s \cdot 100$	Silica gel No. 1	Silica gel No. 2	$p/p_s \cdot 100$	Silica gel No. 1	Silica gel No. 2
	$x/m \cdot 100$			$x/m \cdot 100$			$x/m \cdot 100$			$x/m \cdot 100$	
Ascending points			*Descending points*			*Descending points*			*Descending points*		
21.6	12.3	12.9	49.5	27.0	30.0	18.2	11.2	10.7	61.3	29.4	34.8
29.4	15.9	15.9	40.8	25.6	27.1	12.1	8.4	8.5	42.8	27.0	31.3
36.2	18.7	19.8	36.1	24.3	24.2	*Ascending points*			34.4	24.5	26.9
50.2	26.2	28.4	33.9	22.2	21.7	19.7	11.9	10.4	31.6	21.0	21.3
58.4	28.3	32.6	30.5	18.1	17.5	32.3	17.8	17.5			
			25.2	13.8	14.1	50.2	25.8	27.6			
						85.8	30.9	36.5			

NOTE:—*Time allowed for equilibrium 12 hr. $T = 20.43°$ C.*

The hysteresis has not disappeared though its magnitude is slightly reduced. However, it will be observed that in the first cycle the highest pressure attained was 59% of saturation, and the descending points from this maximum fall within the hysteresis loop of the second cycle which was carried to a higher value, offering a further indication of the dependence of hysteresis on the manner of addition or withdrawal of vapor.

Apparently the pressure changes have not been great enough to affect the hysteresis appreciably. This was perhaps to be expected as the sorption balance, owing to the small samples which may be examined, has a much lower ratio of gel volume to apparatus volume than most of the common methods which are employed to examine sorption. (In Patrick's apparatus 1200 cc. of silica gel was initially contained in an apparatus whose volume was prob-

ably little greater than that employed in the present case where the samples totaled some 2 gm.). Steps were accordingly taken to make the conditions in this respect more comparable. A single spiral-tube was employed and 20 gm. of silica gel was placed in the bottom of the tube under the sample. With this additional sorbent in the system the amount of water vapor taken up between different points on the isotherm was about one gram. The distillation of this weight of water vapor into the system entailed a considerable rise in vapor pressure which was maintained for some time, and which later fell to the equilibrium value after closing off the water tube. In these experiments the samples were not heated during evacuation, as this process had been shown to have no effect on the results. The samples were evacuated and "washed" with water vapor as previously described.

The results appear in Table IV, together with the amount of pressure change taking place during the establishment of equilibrium at various points. In obtaining the descending points it was possible to lower the pressure to the order of a few millimetres of mercury, by operating the pump at its usual speed. When the system was isolated the pressure rose through the range indicated, as the sample lost water. It is quite apparent that the outside of the gel would become dried during the pumping process and in the later stages would approach the equilibrium by *gain* of water. There is evidence that similar conditions held in Patrick's experiment, as it is stated that "The partial pressure of water vapor over the saturated gel was reduced somewhat by pumping."

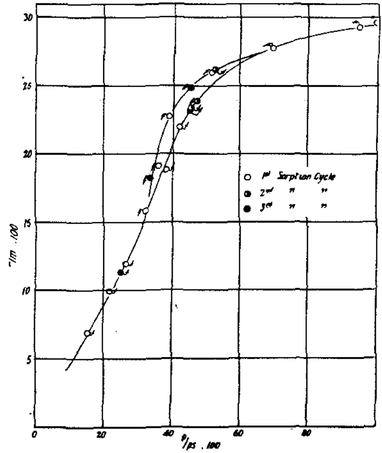

FIG. 5. *Sorption of water by silica gel; large pressure changes taking place during sorption.*

The results of Table IV appear in Fig. 5. It is apparent that the magnitude of the hysteresis has been greatly reduced, while certain points in the region of the loop are reversible, and in one case the position of "ascending" and "descending" points is reversed. While the loop has not disappeared completely in all cases, it seems fairly definite that under the correct conditions the hysteresis would become smaller than the experimental error.

TABLE IV

RESULTS OBTAINED WITH VARIABLE VAPOR PRESSURE (LARGE PRESSURE CHANGES)

p/p_s . 100	x/m silica gel	p/p_s . 100	x/m silica gel	Press. change, in mm.	p/p_1 change, %	p/p_s . 100	x/m silica gel	p/p_s . 100	x/m silica gel
Ascending points			*Descending points*			*Ascending points*		*Ascending points*	
15.7	6.9	95.0	29.3	12.2	70	40.1	20.3	25.0	11.3
21.9	9.9	69.7	27.8	9.0	52	45.5	23.4	45.8	23.4
26.3	11.9	51.9	26.0	8.0	46	52.9	26.2	100	30.1
38.3	18.8	39.0	22.7	6.6	38	*Descending points*		*Descending points*	
42.2	21.9	35.9	19.1			47.4*	23.9	45.6	24.9
100.0	29.6	32.5	15.8					33.7	18.2
								Ascending point	
								42.2	22.2

NOTE:—*Time allowed for equilibrium 12 hr. $T = 20.00°$ C.*
* *Pressure change, 8 mm.; p/p_1 change, 46%.*

Conclusions

It appears from the above that the hysteresis loop which appears during water sorption by silica gel has a real existence and is not due to the failure to eliminate air and other gases from the system. This is in accord with the findings of Anderson and other early workers on the subject, who did not take elaborate precautions to remove air from their sorption systems, and also those of Urquhart and Lambert and coworkers who carefully evacuated their samples. The reason for the disappearance of the hysteresis in the one case where it has not been reported may be due to the large pressure changes which were allowed to take place during the establishment of equilibrium, and which were of such a nature as to obscure the hysteresis. It has been possible to obtain a repetition of this behavior to a certain extent and alter the dimensions of the loop.

While from a theoretical point of view it is obvious that in any system showing hysteresis great pains must be exercised to ensure that the whole system approaches the equilibrium strictly in the direction indicated, it has been shown that very large pressure changes are required to appreciably affect the dimensions of the hysteresis—so that in most cases the precautions that were taken in this research to ensure constant pressures would not be essential.

II. SORPTION OF BENZENE AND ETHYL ALCOHOL BY SILICA GEL

It has been mentioned in the introduction that Anderson found hysteresis loops during the sorption of benzene vapor by silica gel, while Lambert and Clark later obtained reversible isotherms without trace of hysteresis. It has been suggested that this difference was due to the incomplete elimination of inert gases in the original experiments. Patrick, on the other hand, obtained reversible isotherms in the presence of air* using a dynamic method.

The sorption of benzene and ethyl alcohol vapors has been carried out in the sorption balance without taking the elaborate precautions which have been recommended by Patrick and others. Samples of commercial silica gel were evacuated at room temperature after which benzene vapor was added, followed by further evacuation, this succession of events being repeated twice.

In the experiments with alcohol the gel samples were not "washed" with vapor, but were simply evacuated until no gas was given off.

The apparatus described previously (Fig. 1) was employed. The vapor pressure was not absolutely constant during sorption but the interposition of a two litre flask in the system made it nearly so, as the volume of sample employed was not greater than 2 cc., while the total volume of the apparatus was about four litres. The work on water having shown that fairly large pressure changes are required to affect a hysteresis appreciably, this degree of pressure constancy seemed adequate.

The results of these experiments appear in Tables V and VI and are plotted in Fig. 6. No hysteresis appeared in either case and the curves are in general similar to those reported by Lambert and coworkers. The benzene isotherm is notably different from that of water, in that most of the sorption takes place at low relative vapor pressures. Alcohol appears to occupy an intermediate position. The differences have been referred to by Lambert and others and will not be discussed further here. It seems quite definite that no hysteresis appears during the sorption of these vapors by silica gel and its absence is not connected with the removal of traces of inert gases, as has been suggested. Anderson reported loops for both of these sorbents which were similar in shape to those found for water but they have not been repeated in any later work.

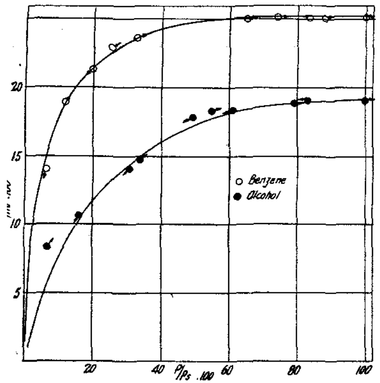

FIG. 6. *Sorption of vapor by silica gel. Isotherm bars—benzene at 20° C., and alcohol at 23° C.*

TABLE V

SORPTION OF BENZENE BY SILICA GEL (T = 20.04° C.)

Silica gel No. 1						Silica gel No. 2			
p/p_s.100	x/m.100	p/p_s.100	x/m.100	p/p_s.100	x/m.100	p/p_s.100	x/m.100	p/p_s.100	x/m.100
Ascending points		*Descending points*		*Ascending points*		*Ascending points*		*Descending points*	
32.8	23.6	83.5	25.0	11.9	18.5	31.0	23.6	43.2	25.0
65.0	25.0	19.9	21.3	25.2	22.9	45.0	25.2	15.6	21.0
74.0	25.1	6.1	14.0	65.8	24.6	62.3	25.4	*Ascending points*	
100.0	25.1			88.0	25.0	77.2	25.6	46.5	24.8
						84.0	25.7	65.3	25.3

TABLE VI

SORPTION OF ALCOHOL VAPOR BY SILICA GEL (T = 23.00° C.)

	Ascending points					Descending points				
p/p_s . 100	6.5	33.8	54.9	79.3	100.0	83.3	61.5	48.8	30.7	16.0
x/m . 100	8.3	14.7	18.2	18.8	19.0	19.0	18.3	17.8	14.1	10.6

Conclusions

It has been demonstrated that the hysteresis which appears during the sorption of water by silica gel may not be removed by special methods of evacuation and is therefore not due to the presence of permanent gases in the system. On the other hand, McGavack and Patrick have shown that when air is present in the sorption of sulphur dioxide by silica gel, a hysteresis definitely appears which may be removed by elimination of the air.

In order to seek an explanation of the apparent contradiction the results of McGavack and Patrick have been plotted as x/m against p/p_s, rather than against pressure. A typical isotherm appears in Fig. 7 together with one from the work of Lambert and Foster on water. It is at once apparent that when the results are plotted on the same basis, there is not necessarily any relation between them, and that an explanation suitable for one may not be applicable to the other.

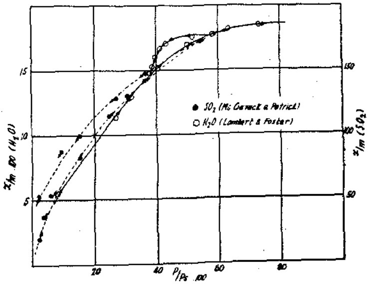

FIG. 7. *Sorption of sulphur dioxide and water by silica gel.*

These general types of hysteresis loop have been discussed by Urquhart who points out that the sulphur dioxide desorption and sorption curves are identical, but are separated by a constant distance in a direction parallel to the pressure axis, while the water hysteresis loop encloses an area of characteristic shape. Again the sulphur dioxide hysteresis extends from the origin as far as the isotherm was examined, and hence over a region where other authors obtained perfect reversibility. It is difficult to see why, if the cause of hysteresis in the water system is sorbed air, it should exert its effect in a specific portion of the isotherm. It seems that these two effects are quite separate and it is unfair to compare the two isotherms.

The comparison would probably not have been made if the isotherms had been compared only over the same portion, as in Fig. 7. That is, when the sorption of different vapors below the critical temperature are to be compared, the sorption values should be plotted against p/p_s rather than pressure. In this way it is possible to compare the amount of sorption taking place at the same fraction of the saturation pressure in the case of different vapors.

A number of other cases could be cited where a failure to emphasize this point has lead to doubtful conclusions. Patrick, Preston and Owens (13), for example, examined the sorption of carbon dioxide and nitrous oxide on silica gel. In the interests of the capillary condensation theory they investigated the sorption of these vapors above and below the critical temperature, and obtained no break in the curves. They concluded therefore that "in the pores of the gel, the critical temperature is raised, and that condensation and surface tension exist even at 40°". Actually these experiments were carried out at pressures of 650 mm. while p_s was of the order of 70 atmos. The ratio p/p_s was therefore approximately 0.01 and it is generally considered that the capillary condensation theory may not be applied below $p/p_s = 0.1$ even if it is applicable at higher pressures.*

Urquhart quotes the work of Gregg (3) in this connection, pointing out the same error. This author examined the sorption of nitrogen, carbon dioxide, carbon monoxide, acetylene, ethylene, ethane and ethyl chloride; a permanent hysteresis was found only in the last case, which was then considered to be anomalous and the results were discarded. Plotting these results against p/p_s (excluding, of course, gases above their critical temperature) it will be seen that widely different portions of each isotherm were examined and only in the case of ethyl chloride was the saturation pressure closely approached—there is therefore no assurance that hysteresis might not have occurred in some other case, had comparable values of p/p_s been obtained.

In general it seems that the phenomenon is too complicated to be disposed of with the simple suggestion which has been put forward, and no entirely satisfactory explanation for its appearance or disappearance in sorption by inorganic materials has been put forward.

* *McBain and Britton (8) examining the effect of critical temperature on sorption of the nitrous oxide by charcoal, employed pressures up to 60 atmos., bringing p/p_s to reasonable limits.)*

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THE HARSHNESS OF WOOL AND ITS MEASUREMENT[1]

By P. Larose[2]

Abstract

As part of an investigation into the causes of the harshness of certain wools, an apparatus was devised by means of which the ease or extent to which wool can be compressed is measured. Four samples of yarn differing in harshness have been tested with this apparatus. The same samples were also tested by a method described by Winson. The results show that when the wools are placed in order of decreasing harshness, they are also in order of increasing compressibility; the softer the wool the more easily it is compressed.

Measurements have been made at 50% and at 60% relative humidity.

Introduction

The present study was undertaken as a result of an enquiry relating to the cause of the harshness in some wools when compared with other wools of the same fineness when spun into yarn. This difference in harshness seemed more pronounced when the wool was dyed.

The first problem was to devise a method which would permit the comparison of the harshness of various wools and to a certain degree its expression in quantitative terms. The author believes that the method described in this paper answers the purpose sufficiently well to serve as a measure of harshness.

Since the feeling of harshness is a tactual one obtained on compressing the wool in the hand or between the fingers, it seemed reasonable to suppose

The yarns on the study of which the present communication is based were 4/14's of 56's quality. They were made from two different batches of wool and the yarn from one was found to be harsher than that of the other although they had undergone the same treatment in the mill as regards twisting, dyeing, etc. After dyeing, the yarn was still harsher. Four samples were consequently examined; one white and one that was dyed red, from each of the two wools. Unfortunately the source of the wool could not be traced and the breed of sheep from which the wool of one batch originated may not have been the same as that from which the other batch was obtained. The samples had however been selected by practical mill men as representing a harsh wool and a relatively soft one respectively. Moreover in the judgment of these men, the dyed yarn was in each case somewhat harsher than the corresponding white yarn. This was readily confirmed by tactual examination when the samples arrived at the laboratory.

For future reference the harsher of the two wools will be called No. 1, and the other or softer wool will be known as No. 2.

The present work will be continued with a view to correlating the results obtained by this method with other elastic properties of the wool fibre, and to determine the effect of various manufacturing operations on this property.

Apparatus

The apparatus used in the first method was the same as that described by Winson (4). The rubber balloon had a capacity of about 50 cc. and the wool was subjected to pressures up to 50 cm. of mercury.

The apparatus devised for the second method is shown in Fig. 1. It consists of a rod fixed to a solid base and on which slide three supports or brackets, *A*, *B* and *S*. These can be fixed in position by means of screws *Y*. Support *S* holds a circular knurled nut *F* threaded to receive the screw *E*, an opening in *S* being large enough to allow the screw *E* to slip through. A cylinder *C* is fixed rigidly to the screw *E* and into it is placed the sample to be tested. The actual dimensions of the cylinder used are 4.4 cm. diameter and 3.72 cm. depth. By turning the nut *F* the screw *E* with cylinder can be raised or lowered. The pitch of the screw is 1 mm. and *F* is graduated near the edge into 100 divisions so that the displacement of the cylinder can be measured to 0.01 mm. A small pointer *N* fixed to *S* enables one to read fractional turns of the nut *F* while the number of com-

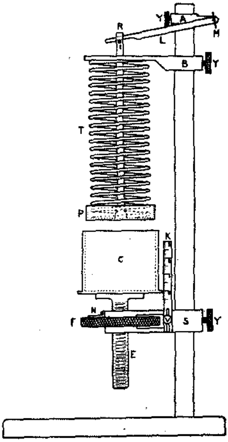

FIG. 1. *Apparatus for measuring compressibility of wool.*

plete turns are determined by noting the movement of the cylinder along scale K graduated in mm. and fixed to S by means of screw V.

The support B holds a spring T, flattened at both ends. Through B passes the rod R of the piston P which is of aluminium so as to be as light as possible. The outside diameter of the piston is a little less than the inside diameter of the cylinder in order to eliminate all friction between the two. The upper part of the rod R is slit so as to permit a thin lever L to rest on a pin I fixed through the piston rod. At the other end of the lever is rigidly fixed a pin which is free to rotate in the support A. To the pin is fixed a small mirror M with its plane parallel to the pin and at right angles to the lever. A spot of light thrown on this mirror and reflected on to a scale some distance away served as the means of determining the displacement of the piston and the compression of the spring with which the piston was in contact throughout the experiment.

The apparatus as described above involves no new principle. A helical spring for exerting the pressure has been used by Schiefer (3), and a piston and a cylinder formed part of an apparatus described by Haven (1). Haven's apparatus however was designed for large quantities of material, and that of Schiefer is not suitable for fibrous material in loose form. The present apparatus is practically free of the frictional error found by Schiefer with his apparatus, and has also been used successfully with asbestos by van Winsen*, and with other fibrous material.

Experimental Procedure

The experiments carried out by Winson's method were made under ordinary room conditions, but the experiments by the cylinder method were performed in a room where the relative humidity was maintained at 50% for one series of tests and at 60% for another, and the temperature at 70° F.

The sample of wool to be tested was placed in the cylinder after the latter had been moved to its lowest point near the zero of the scale K. The support S was then raised gently until the wool touched the piston which hung away from the spring, the pin L through the piston rod preventing the piston from dropping through the support B. The cylinder was raised further until the piston almost touched the spring. The position of the piston, as indicated by the spot of light, when in contact with the spring was determined previously by pushing up the piston lightly with the finger. The resistance offered by the spring the moment the two are in contact is readily felt by the finger. This gives the zero position for the spot of light and when once determined supports A and B should not be changed. The nut F is then set at the zero point and held there while the cylinder is rotated until the piston touches the spring, that is, until the spot of light reaches its zero position. The scale K is then so adjusted that its zero point coincides with some reference mark on the cylinder.

The apparatus is now ready for the compression of the material to be tested. It will be noted that the wool is already slightly compressed by the

* *Unpublished results.*

piston but the latter was made so light (15 gm.) that the error in the zero volume obtained by extrapolation is very small. From now on, the nut *F* is slowly rotated to raise the cylinder. It may be necessary occasionally to hold the cylinder to prevent it from rotating with the nut. At various intervals during the experiment the position of the spot of light on the scale is noted, while the displacement of the cylinder is also observed. This displacement is readily obtained by noting the position of the cylinder along *K*, and the division on *F* opposite the index *N*. In the actual experiments, a reading was taken for every 5 cm. movement of the spot of light until the total displacement was 55 cm., which corresponded to a spring compression of 1.33 cm. Previous calibration of the spring showed this to correspond to a pressure of 761 gm. for the tests at 60% relative humidity, and 792 gm. for those carried out at 50% relative humidity. The release stroke was then performed in the reverse direction, the nut being turned so as to lower the cylinder.

As in the first method described by Winson, for the first few strokes the wool does not come back to its original volume after the pressure is released, and it becomes necessary after each stroke to adjust the support *S* and the cylinder in order to bring the piston in contact with the spring and start from the same zero point. Generally this becomes unnecessary after the fifth or sixth stroke. In order to calculate the actual volume occupied by the wool the distance between the top of piston and bottom of cylinder had to be measured with a cathetometer once during each cycle, generally at the beginning.

The form of the samples used in the two methods was not the same. In the first method the yarn was cut in lengths of 2½ in. and the four strands were separated from one another. The method having been devised for raw wool it was thought that this procedure would, without undue work, give the wool such a form that the tests with it would approximate as nearly as possible the tests with raw wool. In the second method however no such division of the yarn was made. The necessary length of yarn, weighing about three grams, was simply folded over and over until it formed a small bundle about 2½ in. long. This was placed in the cylinder with the yarn approximately parallel to the bottom. However, care was taken that the yarn was as nearly as possible in the same condition in all experiments. For instance, the length of yarn and method of folding were exactly the same in all tests, so that the small bundle was always made up of the same number of strands.

Results

Before carrying out any compressibility tests the main dimensional characteristics which would possibly affect this property were determined. These were fibre length, fibre diameter and number of scales. The results obtained are shown in Table I. Only the undyed yarn was used.

TABLE I
DIMENSIONAL CHARACTERISTICS

—	Fibre diam.	Fibre length, cm.	Scales per mm.
No. 1	30.4μ	9.8	76
No. 2	29.9μ	12.7	72

Table II illustrates a typical set of observations obtained in the compressibility experiments with a sample of No. 2 dyed yarn at 50% relative humidity. The weight of wool was 3.28 gm. The observations for the compression stroke only are recorded.

TABLE II

A TYPICAL SET OF OBSERVATIONS

Scale reading for piston displacement, cm.	Cycle I	Cycle II	Cycle III	Cycle IV	Cycle V	Cycle VI	Cycle VII
	Cylinder displacement, cm. (given by micrometer screw)						
5	0.22	0.24	0.24	0.23	0.23	0.22	0.22
10	0.47	0.48	0.46	0.46	0.46	0.45	0.45
15	0.73	0.73	0.70	0.70	0.68	0.68	0.69
20	0.95	0.96	0.93	0.93	0.92	0.90	0.91
25	1.15	1.17	1.15	1.15	1.13	1.13	1.13
30	1.37	1.37	1.34	1.34	1.32	1.32	1.30
35	1.56	1.56	1.53	1.54	1.50	1.52	1.49
40	1.76	1.75	1.71	1.71	1.69	1.68	1.67
45	1.94	1.94	1.88	1.89	1.86	1.86	1.85
50	2.11	2.10	2.06	2.06	2.04	2.01	2.02
55	2.28	2.28	2.22	2.23	2.20	2.18	2.18
Top of piston to bottom of cylinder before compression	14.41	14.39	14.32	14.31	14.26	14.26	14.26

TABLE III

RESULTS AT 50% HUMIDITY FOR THE FOUR SAMPLES OF YARN.
The figures are based on 3.25 gm. of material

Press., gm.	No. 2 Undyed		No. 1 Undyed		No. 2 Dyed		No. 1 Dyed	
	Vol. (V), cc.	Ratio $\frac{V}{V_0}$	Vol. (V), cc.	Ratio $\frac{V}{V_0}$	Vol. (V), cc.	Ratio $\frac{V}{V_0}$	Vol. (V), cc.	Ratio $\frac{V}{V_0}$
Down stroke								
15	30.6	0.974	37.7	0.987	35.6	0.984	43.4	0.987
67	28.1	.895	35.8	.937	33.4	.923	41.9	.952
132	25.6	.816	34.0	.890	31.7	.876	39.8	.905
202	23.8	.758	32.2	.843	29.7	.821	38.1	.866
272	22.5	.716	30.7	.804	28.3	.782	36.7	.834
342	21.3	.678	29.3	.767	27.0	.746	35.5	.807
415	20.5	.653	28.2	.738	25.9	.716	34.3	.780
488	19.6	.624	27.2	.712	25.0	.691	33.2	.754
562	19.1	.608	26.5	.694	24.2	.666	32.2	.732
638	18.5	.589	25.8	.676	23.5	.649	31.5	.716
716	18.0	.574	25.2	.660	23.0	.636	30.7	.698
792	17.6	.561	24.5	.641	22.4	.619	30.0	.682

TABLE III—*Concluded*

RESULTS AT 50% HUMIDITY FOR THE FOUR SAMPLES OF YARN.
The figures are based on 3.25 gm. of material

Press., gm.	No. 2 Undyed		No. 1 Undyed		No. 2 Dyed		No. 1 Dyed	
	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$
Up stroke								
716	17.6	.561	24.6	.644	22.4	.619	30.1	.684
638	17.7	.564	24.7	.647	22.4	.619	30.2	.687
562	17.9	.570	24.8	.649	22.5	.622	30.3	.689
488	18.0	.574	25.1	.657	22.6	.624	30.8	.700
415	18.5	.589	25.4	.665	22.9	.633	31.1	.707
342	19.0	.605	25.9	.678	23.4	.646	31.7	.721
272	19.6	.624	26.7	.699	24.2	.668	32.5	.739
202	20.5	.653	27.5	.720	25.2	.696	33.2	.754
132	22.0	.701	29.0	.759	26.7	.738	35.2	.798
67	24.7	.787	32.0	.838	29.5	.815	38.1	.866
V_o	31.4		38.2		36.2		44.0	

TABLE IV

RESULTS AT 60% HUMIDITY FOR THE FOUR SAMPLES OF YARN.
The figures are based on 3.25 gm. of material

Press., gm.	No. 2 Undyed		No. 1 Undyed		No. 2 Dyed		No. 1 Dyed	
	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$	Vol. (V), cc.	Ratio $\frac{V}{V_o}$
Down stroke								
15	33.0	0.982	37.3	0.992	36.0	0.986	40.9	0.993
64	30.9	.920	36.0	.957	34.5	.945	39.2	.952
127	28.6	.852	34.7	.923	32.8	.898	37.9	.920
194	26.6	.792	33.4	.888	31.0	.849	36.6	.888
262	24.9	.741	32.2	.856	29.9	.819	35.4	.859
329	23.6	.702	31.0	.824	28.6	.784	34.4	.835
400	22.3	.664	29.9	.795	27.3	.748	33.3	.808
470	21.5	.640	29.1	.774	26.3	.720	32.4	.786
541	20.6	.614	28.4	.755	25.5	.698	31.8	.772
614	19.9	.592	27.5	.731	24.5	.671	31.0	.753
689	19.5	.581	27.0	.718	23.9	.655	30.4	.738
761	18.9	.563	26.3	.699	23.4	.641	29.8	.724
Up stroke								
689	19.2	.572	26.4	.702	23.5	.644	29.9	.726
614	19.4	.578	26.6	.707	23.7	.649	30.0	.728
541	19.5	.580	26.8	.712	23.9	.655	30.1	.730
470	19.7	.586	27.0	.718	24.2	.663	30.4	.738
400	20.1	.598	27.2	.723	24.5	.671	30.7	.745
329	20.5	.610	27.6	.734	25.2	.690	31.1	.755
262	21.5	.640	28.1	.747	25.8	.707	31.7	.770
194	22.5	.670	29.0	.771	27.0	.740	32.6	.792
127	24.3	.724	30.3	.802	28.6	.784	33.9	.823
64	27.2	.810	32.5	.864	31.2	.855	36.5	.886
V_o	33.6		37.6		36.5		41.2	

TABLE V

RESULTS OF TESTS BY WINSON'S METHOD

No. 1 Dyed			No. 1 White			No. 2 Dyed			No. 2 White		
Press., cm. Hg.	Vol., cc.	β/β_o	Press., cm. Hg.	Vol., cc.	β/β_o	Press., cm. Hg.	Vol., cc.	β/β_o	Press., cm. Hg.	Vol., cc.	β/β_o
Down stroke											
0.0	43.1	1.00	0.0	40.2	1.00	0.0	40.6	1.00	0.0	37.6	1.0
2.57	40.6	.941	4.76	33.9	.844	4.73	34.7	.853	4.28	31.7	.84
5.33	37.6	.873	10.69	29.0	.721	10.59	29.8	.732	10.18	26.7	.73
8.40	35.2	.817	12.29	28.2	.702	17.06	26.7	.658	16.35	23.7	.64
11.07	32.7	.760	16.80	26.3	.656	23.83	24.6	.606	22.70	21.8	.59
14.50	31.8	.737	24.30	24.7	.614	30.08	23.3	.573	29.09	20.4	.554
18.00	29.8	.692	27.18	24.0	.598	38.00	22.1	.544	37.21	19.1	.524
21.04	29.0	.673	31.13	23.3	.579	45.22	21.2	.521	44.04	18.2	.50
24.81	27.7	.643	38.64	22.1	.550	50.76	20.7	.510	47.28	18.0	.490
27.91	26.9	.625	45.72	21.2	.527				52.65	17.0	.452
34.60	25.4	.590	50.94	20.6	.514						
41.70	24.3	.564									
50.51	22.8	.534									
Up stroke											
41.28	23.1	.538	45.28	20.7	.515	44.60	20.7	.510	44.35	17.5	.465
34.08	28.6	.552	38.21	20.9	.520	34.20	21.4	.527	37.44	17.7	.470
30.24	23.6	.548	36.91	21.1	.525	29.50	21.6	.531	29.33	18.2	.483
26.68	24.4	.573	30.68	21.5	.535	23.70	22.4	.551	21.66	19.1	.509
22.85	24.8	.575	23.40	22.3	.556	19.04	23.3	.574	19.46	19.6	.522
19.59	25.7	.601	20.47	23.0	.574	15.86	23.9	.590	15.23	20.3	.541
15.89	26.4	.613	16.27	23.8	.592	13.38	24.9	.613	8.79	23.0	.612
12.47	28.0	.656	9.65	26.2	.652	9.47	26.2	.646	3.38	28.4	.755
9.73	28.9	.670	6.95	28.1	.700	6.33	28.7	.706	1.21	33.4	.890
6.16	32.1	.745	3.89	30.9	.770	3.94	31.5	.774	0.03	36.4	.970
3.83	34.5	.800	0.07	38.8	.966	1.53	36.2	.891			
0.89	40.1	.920									

The ratio $\frac{V_p}{V_{15}}$ was calculated instead of the ratio $\frac{V_p}{V_o}$. V_o was not determined as it would have to be obtained by extrapolation of the curves, which were not drawn in these cases. However the difference that would result from such a change would be very small.

The results in Tables III and IV are plotted in Figs. 2, 3, 4 and 5. In Fig. 2 are plotted the volumes occupied by the wool at various pressures and 50% humidity, and in Fig. 3 are similar results at 60% humidity. The thick lines represent the compression strokes and the light lines, the release or up-strokes. In Fig. 4 are plotted the volumes for the compression strokes only, but for the two humidities for purposes of comparison. Fig. 5 gives the ratios of the volumes at different pressures to the original volume, V_o, for the compression strokes. In Figs. 4 and 5 the dotted lines represent the results at 50% humidity, and the full lines, the results at 60% humidity.

In Table V and Figs. 6 and 7 are given the results obtained by the balloon method. In Fig. 6 the results are plotted as in Figs. 2 and 3, while in Fig. 7 they are plotted as by Winson. It is to be noted that Winson corrected for

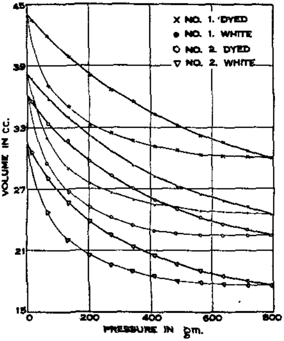

FIG. 2. *Relation between volume and pressure at 50% humidity.*

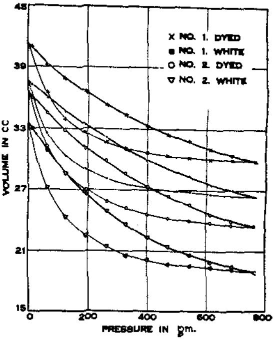

FIG. 3. *Relation between volume and pressure at 60% humidity.*

the actual volume of the wool which was subtracted from the total volume occupied by the wool to obtain the free volume. This was not done in giving the results in the above tables (Table V excepted) and in plotting Figs. 2, 3, 4 and 5. It was found that there was no advantage in doing this for purposes of comparison.

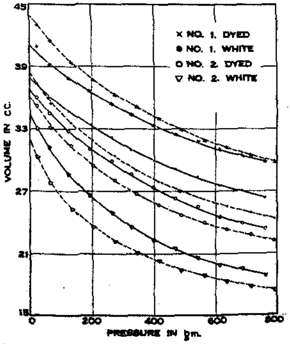

FIG. 4. *Relation between volume and pressure for down stroke. The full lines represent the results at 60% humidity, and the broken lines the results at 50% humidity.*

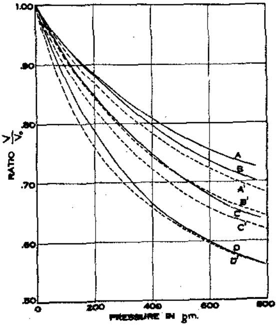

FIG. 5. *Relation of V/V_0 to pressure for down stroke. The full lines represent the results at 60% humidity, and the broken lines the results at 50% humidity. A and A', No. 1 dyed; B and B', No. 1 white; C and C', No. 2 dyed; D and D', No. 2 white.*

TABLE VI

COMPARISON OF VOLUMES OCCUPIED BY THREE DIFFERENT WEIGHTS OF WOOL (No. 2 DYED)

Press., gm.	3.26 gm.			4.34 gm.				2.20 gm.			
	V_p, cc.	$\frac{V_p}{\text{Weight}}$	$\frac{V_p}{V_{15}}$	V_p, cc.	$\frac{V_p}{\text{Weight}}$	$\frac{V_p}{V_{15}}$	Vol. calcd. to 3.26 gm.	V_p, cc.	$\frac{V_p}{\text{Weight}}$	$\frac{V_p}{V_{15}}$	Vol. calcd. to 3.26 gm.
Down stroke											
15	35.7	1.10	1.00	47.1	1.09	1.00	35.4	28.0	1.27	1.00	41.5
67	33.5	1.03	0.94	44.5	1.03	0.95	33.5	25.8	1.17	0.92	38.2
132	31.8	0.98	0.89	42.0	0.97	0.89	31.6	23.1	1.05	0.83	34.2
202	29.8	0.91	0.84	39.7	0.92	0.84	29.8	21.3	0.97	0.76	31.6
272	28.4	0.87	0.80	37.7	0.87	0.80	28.3	19.9	0.90	0.71	29.5
342	27.1	0.83	0.76	35.9	0.83	0.76	27.0	18.9	0.86	0.68	28.0
415	26.0	0.80	0.73	34.5	0.80	0.73	25.9	17.9	0.81	0.64	26.5
488	25.1	0.77	0.70	33.1	0.76	0.70	24.9	17.2	0.78	0.61	25.5
562	24.3	0.75	0.68	32.1	0.75	0.68	24.2	16.6	0.75	0.59	24.6
638	23.6	0.72	0.66	31.2	0.72	0.66	23.5	16.1	0.73	0.58	23.8
716	23.1	0.71	0.65	30.4	0.70	0.64	22.9	15.5	0.71	0.55	23.0
792	22.5	0.69	0.63	29.8	0.69	0.63	22.4	15.1	0.69	0.54	22.4
Up stroke											
716	22.5	0.69	0.63	29.8	0.69	0.63	22.4	15.1	0.69	0.54	22.4
638	22.5	0.69	0.63	29.9	0.69	0.64	22.5	15.2	0.69	0.54	22.5
562	22.6	0.69	0.63	30.1	0.69	0.64	22.6	15.4	0.70	0.55	22.8
488	22.7	0.70	0.64	30.4	0.70	0.65	22.9	15.5	0.71	0.55	23.0
415	23.0	0.71	0.64	30.9	0.71	0.66	23.2	15.8	0.72	0.56	23.4
342	23.5	0.72	0.66	31.3	0.72	0.66	23.5	16.3	0.74	0.58	24.2
272	24.3	0.75	0.68	32.2	0.74	0.68	24.2	16.9	0.77	0.60	25.0
202	25.3	0.78	0.71	33.7	0.78	0.72	25.4	17.8	0.81	0.64	26.4
132	26.8	0.82	0.75	35.6	0.82	0.76	26.8	19.3	0.88	0.69	28.6
67	29.6	0.91	0.84	39.8	0.92	0.85	29.9	21.9	1.00	0.78	32.5

Example of Calculation

Taking the last reading of Table II for example, the cylinder was raised through 2.18 cm. while the piston's displacement was that corresponding to the 55 cm. of the scale. Calibration of the scale with the cylinder empty showed that 55 cm. of the scale was equivalent to a piston movement of 1.33 cm. The amount of compression then was $2.18-1.33$ or 0.85 cm. Since the height of the piston was 11.92 cm., the original height of the wool was $14.26-11.92$ or 2.34 cm. and after compression, $2.34-0.85=1.49$ cm. The area of the cylinder being 15.2 sq. cm., the volume of the wool after compression was $15.2 \times 1.49=22.6$ cc. The pressure at which the wool had this volume was obtained from the piston displacement, which was the same as the spring compression, and a previous calibration of the spring. The pressure for the above figures was 792 gm.

These calculations need not be done with all the readings, but only on the means for the cycles carried out after the wool has apparently reached an equilibrium, such as the last three cycles in the example given above.

Since all the samples differed slightly in weight, a correction had to be made in order to compare the results. For this correction it was assumed that the volume occupied by the wool was proportional to the weight, at least within the narrow range where the correction was applied. This was justified by some tests made with three different weights of the No. 2 dyed sample. The results of these tests are given in Table VI. The other results are given in Tables III and IV. As the weights of the samples varied around 3.25 gm. all the figures have been calculated for this weight of wool. The results are the means of three or more determinations on different samples.

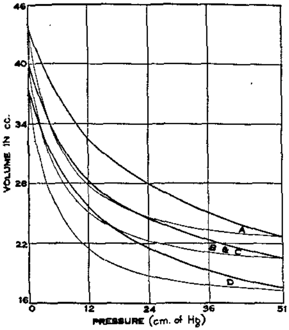

FIG. 6. *Relation between volume and pressure (Winson's method). A, No. 1 dyed; B, No. 1 white; C, No. 2 dyed; D, No. 2 white.*

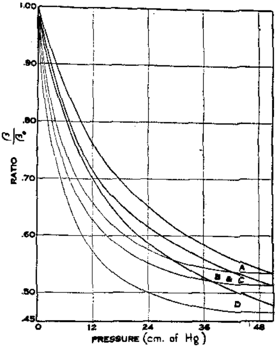

FIG. 7. *Relation between β/β_0 and pressure (Winson's method). A, No. 1 dyed; B, No. 1 white; C, No. 2 dyed; D, No. 2 white.*

Discussion

Since the samples used in the two methods were not of the same form, direct comparison is probably not justified. However the two sets of results as represented by the curves should be comparable qualitatively. It is difficult to see for example why the first method showed no difference between No. 1 undyed and No. 2 dyed yarns, while the second showed a pronounced difference. The fact that the relative humidity was not controlled in the first case may account for this. It is possible that these two samples had a different water content owing to different surrounding conditions. The cylinder method has the decided advantage over the other method that the samples can be tested under strictly comparable conditions which can be varied at will within wide limits. It is evident that in the balloon method, since the pressure of the atmosphere over the wool varies during the experiment, the relative humidity and consequently the regain of the wool will also vary. The cylinder method, being carried out at constant pressure, is

not subject to this drawback, and it has also been found to be more convenient. The experiments are more rapidly carried out and the calculations are somewhat simpler. The initial volume of the wool is also more reproducible.

The balloon method was so modified by Pidgeon and van Winsen (2), working in these laboratories, that experiments could be carried out under constant conditions of humidity, but this involved changes which further complicated the method.

Examination of Table VI, in which results are given for three different weights of wool, shows that the volume of the wool is proportional to the quantity of wool taken. Although the agreement with the smaller weight, 2.20 gm., is not very good at the lower pressures, it is good at the higher pressures, the agreement for the higher weight of 4.34 gm. is excellent for all pressures as compared to the 3.26 gm. sample. As the weights of the various samples taken for the tests varied only between 3.05 and 3.35 gm., the correction as made is justified.

Another correction which it was necessary to make before results could be compared was that due to the different moisture content of the wool at 50% and at 60% humidities. This difference amounted to about 1% of the weight of the wool. In order to compare the results obtained at 50% with those obtained at 60% humidity it was necessary to subtract 1% of the values obtained at 50% humidity, which is equivalent to comparing 3.25 gm. at 60% with 3.22 gm. at 50%.

The softest of the yarns tested as determined by feel was the No. 2 white yarn, followed by No. 2 dyed, while No. 1 dyed was the most harsh of the four. As indicated by the curves the results of the experiments place the yarns in the same order if the softness is associated with ease of compressibility.

The results also show that the area enclosed by the loop formed by the compression and release curves cannot be relied on to give a measure of the harshness. The areas of the four loops in Fig. 3 taken from top down are 119, 121, 127, 111 (in arbitrary units) while in Fig. 2 they are 150, 139, 140, 102. It is evident from these figures that the differences in areas for different samples are not large enough to enable one to use them as a measure of harshness. The experimental error for them also seems to be fairly large.

Examination of the curves shows that in order to compare the harshness or springiness of various wools it is not necessary to perform an entire cycle; it is sufficient to compare the volumes occupied by the wools under identical pressures or to determine under what pressures the wools will have the same volume. It is advisable to use a fairly high pressure as the agreement was always found to be better at the higher pressures but even though it may be better to carry out a full compression stroke, the release or return stroke is certainly not necessary. Attention must be drawn to the results of Winson which, if examined in this way, do not all agree with this view of harshness.

In the case of the super Shropshire wether and hog wools, the volume occupied by the hog wool is smaller than that of the wether wool for the same pressure; in other words it is more compressible and therefore would be softer. But this does not seem to hold in the case of some of the others, notably in the case of the mohair and veld wool where the mohair appears to be more compressible in spite of its greater springiness. It would be highly desirable then to carry out a large number of experiments with a wide variety of samples before deciding on the general applicability of the relation found in the case of the yarns tested.

The effect of humidity on the compressibility cannot be determined sufficiently well from the two series of experiments carried out at 50% and 60% humidity. Although in three of the samples the compressibility was higher at 50% this effect was reversed for the fourth sample which happened to be the harshest, as shown in Fig. 4. Tests at widely differing humidities will have to be made before a definite conclusion can be reached on this point. However the order of the curves for the four samples did not change from one condition to the other, so that for purposes of comparison it does not matter at what humidity the tests are made provided that the conditions are maintained constant during the tests. If Fig. 5, where the ratios of the volumes are plotted instead of the actual volumes, is examined instead of Fig. 4, the effect of humidity is the same on all four samples although the effect on the No. 2 undyed sample appears to be very small, while in Fig. 4 it was the greatest. It is doubtful then which of the two methods of plotting is the most valuable. Further tests would help to decide this point. The method of plotting the ratios to original volume suffers from the disadvantage that an error in determining this initial volume affects all the other points.

It might be questioned whether some of the differences noted in these samples are not due to dimensional attributes. This can hardly be so. The differences in the diameters and number of scales are not large and the difference in length seems to be the only possible cause of variation. But if the results of Winson are applicable here one would expect an opposite effect to that noted, namely that the No. 2 which has the longer fibres, would be the harsher (or less resilient as Winson terms it) of the two. It is probable that the greater length of the fibres in No. 2 did make it appear harsher but taking this into consideration would only increase the differences noted between the two wools. The supposition that was made in the introduction that the harshness was probably due to a greater resistance to compression seems therefore to have been borne out by the results of these experiments.

Schiefer has already proposed the use of resilience as a useful criterion of the "handle" or "feel" of fabrics when they are squeezed between the fingers, although he did not mention specifically harshness or softness.

Winson has used the term resilience to express the property being investigated. It is questionable whether the use of the term in this sense is justified. It would be better to reserve for it the meaning given in mechanics in which

it represents the amount of work which an elastic body is capable of doing in recovering from a stressed condition. Its general use to express the power of recovery of a material to its initial state would also be acceptable. However since the curves given all show the wool to come back to its original volume, applying this definition for resilience would show all the wools to be 100% resilient. The use of the term springiness instead of resilience would be more desirable. It is generally used to mean the capacity of resisting compression or deformation which is actually what is being measured in these experiments.

The use of the term resilience has been discussed by Pidgeon and van Winsen. Since the term is being used more and more in application to textiles it would be highly desirable that a suitable definition be proposed and accepted by all concerned.

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THE DECOMPOSITION OF ETHYL ALCOHOL OVER SOME POLY-COMPONENT CATALYSTS[1]

By E. H. Boomer[2] and H. E. Morris[3]

Abstract

Numerous experiments have been carried out on the decomposition of alcohol, alcohol and water, and alcohol and carbon dioxide mixtures over poly-component catalysts at temperatures up to 500° C. Quantitative data on the gaseous and the liquid products were obtained. The properties of the poly-component catalysts, as evidenced by the simple primary and secondary reactions, have been shown to be qualitatively those of the single components.

Methane can be produced in one or more of several secondary reactions, namely, the decomposition of acetaldehyde, the hydrogenation of carbon oxides and the decomposition of ethylene. Ethane can be produced in one or both of two reactions consisting of auto-oxidation and reduction of the alcohol, or the secondary hydrogenation of ethylene, confirming previous work. Carbon dioxide, in most cases, is formed as a result of the water-gas reaction and the decomposition of carbon monoxide. In other cases its origin is obscure. The results of certain experiments in which carbon dioxide and hydrogen were the major constituents of the off-gas cannot be explained in the same way. Reactions involving ketene decomposition and polymerization, and hydration of alcohol, have been suggested as possible explanations.

Introduction

In two previous papers (7, 8) from this laboratory the catalytic decomposition of ethyl alcohol was discussed, and it was suggested that the reactions occurring are numerous and complex. This paper is a further contribution dealing particularly with the origin of methane, ethane and carbon dioxide in such decompositions. In spite of numerous experimental results, the complexity of the reactions prevents more than a qualitative indication of the reactions involved. Many primary, and even more secondary, reactions are possible, the nature and course of which depend upon the catalyst, conditions of temperature, and space velocity used. Among the gaseous products may be hydrogen, carbon monoxide, carbon dioxide, ethylene, methane and ethane. The liberation of carbon is also possible and a few liquids such as water, aldehydes, esters and polymerization products can be formed. Little has been done in the present work with regard to the composition of the liquid products. Attention has been paid to the origin of methane because of the various possible reactions leading to its formation and to the formation of ethane and carbon dioxide, because of the relatively large amounts of these substances formed under certain conditions.

Literature Review

The formation of methane during the decomposition of ethyl alcohol may occur in several ways. The primary decomposition of alcohol to aldehyde,

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followed by the decomposition of aldehyde to methane and carbon monoxide, is probably the most frequent and largest source of methane. The hydrogenation of carbon monoxide may be mentioned as a second important source. While Razuvaev (40) finds only these two reactions and a third, the direct hydrogenation of ethyl alcohol under pressure, as sources of methane, the present authors believe that several other reactions must be considered in experiments at atmospheric pressure. The reactions that appear worthy of consideration, with relevant references, are as follows:

1. $CH_3CHO = CH_4 + CO$	(46)
2. $CO + 3H_2 = CH_4 + H_2O$	(3, 12, 13, 17, 42)
3. $2CO + 2H_2 = CH_4 + CO_2$	(3, 6, 12, 13, 17, 31)
4. $CO_2 + 4H_2 = CH_4 + 2H_2O$	(3, 17, 31, 51, 52)
5. $4CO + 2H_2O = CH_4 + 3CO_2$	(17, 33, 34)
6. $C + 2H_2 = CH_4$	(10, 11, 29, 36)
7. $n(C_2H_4) = CH_4 + C_2H_6 + C$, etc.	(6, 10, 11, 14, 33, 34, 49, 50)

It is possible that any or all of these seven secondary reactions can be sources of methane. Some, such as 1 and 2, may be considered probable and even established by the elimination of others from the range of possibility.

There have been only two reactions proposed as sources of ethane that require consideration here. Engelder (15) suggested that ethane was formed by the hydrogenation of the primary product, ethylene, when hydrogen was present in the "nascent" state. Bischoff and Adkins (5) believe it more probable that auto-oxidation and reduction of ethyl alcohol give rise directly to ethane as follows:

8. $2C_2H_5OH = CH_3CHO + H_2O + C_2H_6$.

The authors concurred in this belief (7) at one time, but the present work suggests that both reactions occur in proportions that vary with experimental conditions.

The production of carbon dioxide during the decomposition of ethyl alcohol may occur through the operation of several reactions. The following secondary reactions may be listed as possibilities as shown by work done elsewhere:

9. $2CO = CO_2 + C$	(20, 48)
10. $C + 2H_2O = CO_2 + 2H_2$	(12, 13)
11. $CO + H_2O = CO_2 + H_2$	(52)

These are all common reactions to which a great deal of attention has been given, although only type references are made to each. In addition, Reactions 3 and 5 are considered as sources of carbon dioxide.

Sabatier and Mailhe (43) have shown that oxide catalysts may be reduced by alcohol vapor with the formation of carbon dioxide, but in the present work this reaction was impossible, or readily evident by its cessation upon complete reduction.

Brown and Reid (9) have suggested that the carbon dioxide is due to the condensation of acetaldehyde to an ester, which on decarboxylation yields carbon dioxide and a hydrocarbon. The decarboxylation of ethyl acetate has been reviewed by Sabatier (42) and further investigated by Adkins (1)

and found to proceed readily over certain catalysts. Armstrong and Hilditch (2) have reported the presence of ethyl acetate among the liquid products of the reaction of ethyl alcohol over nickel at temperatures above 350° C. As acetic acid has been reported so frequently as another product, it seems quite possible that the ester would be formed in many cases. If this is so, the decarboxylation mentioned above would form another possible source of carbon dioxide.

Lazier and Adkins (22) believe, however, that none of the above mechanisms are satisfactory and emphasize several factors that must be taken into consideration. These include the presence of carbon dioxide itself, the acidic nature of all condensates and the frequent observation of a brown water-insoluble resin. Lazier and Adkins developed a mechanism which will account for all these facts. They suggest that the catalyst employed is dehydrogenating, and a ketene is formed.

12. $CH_3CHO = H_2 + CH_2CO.$

The reaction between this ketene and more of the aldehyde would produce carbon dioxide and an unsaturated hydrocarbon, which would polymerize to produce the resin so often noted.

13. $CH_2CO + CH_3CHO = CO_2 + CH_2CHCH_3.$

The reaction between the ketene and water would produce acetic acid, while a reaction between this ketene and ethyl alcohol yields ethyl acetate. Incidentally they stated without comment that the presence of water in the reactants increased the percentage of carbon dioxide and hydrogen.

Experimental Method

The method of investigation has been described previously (7, 8) in sufficient detail except in one respect. Whereas the apparatus described was designed to handle liquids such as ethyl alcohol or aqueous solutions of ethyl alcohol, certain experiments reported now were carried out on carbon dioxide-ethyl alcohol mixtures. In these experiments the apparatus was modified to permit a controlled and measured supply of gaseous carbon dioxide to a saturator containing alcohol maintained at a definite temperature, the gas mixture from the saturator being conducted to the catalyst chamber.

Results and Discussion

Complete results will not be reported but only those necessary to illustrate the nature of the reaction as far as possible. Many data were obtained apart from those presented that confirmed the conclusions given and were of no interest otherwise. The addition of water or of carbon dioxide to the alcohol vapor entering the catalyst chamber was practised as a convenient method of reducing the space velocity. It has been shown by Gilfillan (16) and also in this laboratory that carbon dioxide has little or no effect on the course of the reactions. Similarly, several reports (2, 19, 22, 41) have shown that water acts largely as an inert diluent, but may exert a repressing effect on the reaction.

The Formation of Methane

As has been indicated in the results of other workers, nickel promoted the aldehydic decomposition of ethyl alcohol; the aldehyde was decomposed to methane and carbon monoxide and the latter further broken down to free carbon and carbon dioxide. The results given in Table I indicate these reactions quite clearly. The catalyst, No. 12, was prepared by the ignition of nickel nitrate on asbestos. A second catalyst, nickel chromate, behaved similarly.

TABLE I

EQUIMOLAR ALCOHOL AND WATER.
Catalyst No. 12. Nickel on asbestos by ignition

Temp., °C.	Composition of gaseous products, % by vol.						
	CO_2	CO	Total, as CO	CH_4	H_2	C_2H_6	C_2H_4
330	0.8	25.7	27.3	28.6	40.9	4.0	Nil
375	19.5	5.5	44.5	42.5	30.0	2.5	Nil
400	21.8	3.4	47.0	50.0	24.7	Nil	Nil
450	23.4	4.4	51.0	48.6	23.6	Nil	Nil
500	20.6	2.0	43.2	45.2	32.2	Nil	Nil

The occurrence of ethane in a reaction such as this has been discussed in an earlier paper (8). It is quite evident from the balances obtained in these experiments that the methane is obtained directly from the decomposition of the aldehyde. The hydrogenation of carbon monoxide or carbon dioxide is inappreciable.

The hydrogenation reaction takes place in the presence of carbon dioxide and catalyst No. 22. This catalyst was prepared by mixing together 4 moles of chromic oxide, 1 mole of zinc oxide, 0.5 mole of alumina and traces of copper powder and sodium carbonate. These were well mixed, ground to a paste, and dried to a very hard catalyst which was treated with hydrogen at 300–350° C. Catalyst No. 24 contained 1 mole of zinc oxide, 1 mole of chromic oxide, 0.5 mole of cupric oxide and a trace of copper powder to promote reduction (33, 34). This catalyst was similarly treated. Catalyst No. 27 was prepared by precipitating together in slightly alkaline solution an equimolar mixture of potassium chromate, nickel

TABLE II

EQUIMOLAR ALCOHOL AND WATER

Catalyst No.	Temp., °C.	Composition of gaseous products, % by vol.				
		CO_2	H_2	CH_4	CO	C_2H_4
22	400	10.0	85.5	2.3	Nil	2.1
	500	14.4	78.0	2.3	Nil	5.3
24	400	9.5	86.2	1.6	Nil	2.7
	500	10.4	84.2	1.6	Nil	3.8
27	350	7.3	91.2	Nil	Nil	1.5
	450	18.8	77.8	Nil	Nil	3.4

nitrate, and ferric nitrate, giving an orange yellow precipitate. The liquid was decanted, and the filtrate washed, dried at 110° C., crushed and reduced as before. The results with these three catalysts are given in Table II, with those obtained with catalyst No. 27 for purposes of comparison. The liquid products were characterized by the occurrence of a high boiling oily fraction.

The fact that these catalysts were all essentially dehydrogenating suggested the production of considerable quantities of aldehyde; the total amount of methane was relatively low and if some of the aldehyde was decomposed, the carbon monoxide was either hydrogenated to methane or decomposed to carbon dioxide and carbon. The latter step is rendered improbable by the fact that very little carbon was observed on the catalyst. The excess of water present would decrease the tendency to hydrogenate carbon monoxide (17). It seems improbable therefore that the aldehyde was decomposed. The decomposition of ethylene, as previously shown, gives carbon and ethane as well as methane and hydrogen. The absence of carbon renders the ethylene an unlikely source of the methane. It should also be noted that the methane would increase as the ethylene increased, if there was any decomposition, and such is not the case. The most reasonable mechanism then, involves the hydrogenation of carbon dioxide, since this reaction is not suppressed by water and both gases are present in large proportions. The relatively large amounts of carbon dioxide occurring concurrently with oily condensates will be discussed later. The results with catalyst No. 27 seem to verify the above conclusions; the aldehyde formed was obviously not decomposed, nor was the ethylene polymerized. This catalyst, however, did not promote the hydrogenation of carbon dioxide with the consequent formation of methane, although a hydrogenating reaction might have been expected.

Catalyst No. 28A was also interesting in its results. It was prepared by depositing copper and chromium on silica gel as previously reported (7) and the results have been given. The methane produced over this catalyst could arise from two sources; the decomposition of aldehyde as in Reaction 1, and the hydrogenation of carbon dioxide as in Reaction 4. It is recognized that the decomposition of ethylene would also lead to an analytical excess of water among the products, but the ratio of ethane to methane is not high enough to allow this as a possible source of methane in the light of the work of other investigators.

Another catalyst, No. 28B, which also deserves mention, was that prepared as above, using nickel in addition to copper and chromium on silica, and prepared in a similar manner. The results are given in Table III. These results seem to offer two sources of methane at 400 and 450° C., the hydrogenation of carbon dioxide and the decomposition of ethylene (Reactions 4 and 7).

TABLE III

EQUIMOLAR ALCOHOL AND WATER.
Catalyst No. 28B—nickel, copper and chromium on silica

Temp., ° C.	Composition of gaseous products, % by vol.				
	CO_2	C_2H_4	H_2	CH_4	CO
350	1.1	56.8	42.1	Nil	Nil
400	3.4	57.8	35.0	3.8	Nil
450	9.3	45.0	36.2	8.4	1.1

The action of the nickel in this catalyst is quite evident, but in view of other results (8) in which nickel was found to be more active than either chromium or copper, it was expected that the hydrogen content of the gases would increase instead of decrease. The solution of this anomaly is offered by the hydrogenation of carbon dioxide which requires four volumes of hydrogen for each volume of methane produced. As the temperature was increased from 350 to 400° C., the ethylene content also increased as would be expected from the above, but at 450° C. there was a surprising decrease, and at the same time the carbon dioxide and methane increased. This seems to suggest that some of the ethylene produced was decomposed, including methane as the main product of the reaction with an inexplicable absence of ethane. The decomposition of the aldehyde was evidently not of large magnitude.

TABLE IV

CARBON DIOXIDE AND ALCOHOL.
Catalyst No. 33A—nickel and zinc oxides

Temp., °C.	CO_2 Total	CO_2 Produced	C_2H_4	H_2	CH_4	CO
350	45.2	10.7	Nil	5.9	45.7	3.2
400	47.8	10.8	Nil	10.4	38.1	3.7
450	56.0	18.2	Nil	11.5	28.9	3.6

Catalyst No. 33A, prepared by the precipitation of nickel and zinc hydroxides from an equimolar solution of their nitrates with sodium hydroxide, followed by thorough washing, was studied using carbon dioxide with the alcohol. The results of these experiments are given in Table IV, in which the second column includes carbon dioxide added to the reactants as calculated from the relative gas volumes.

With this catalyst, the formation of the methane was apparently due to the decomposition of aldehyde and the hydrogenation of the carbon oxides. The large excess of carbon dioxide in the gases appeared to favor the formation of methane. The decrease in the percentage of methane was probably due to a decrease in the activity of the catalyst for this hydrogenation reaction, and may be only relative owing to the fact that more of the aldehyde was decomposed, involving the ultimate formation of carbon dioxide as evi-

TABLE V

EQUIMOLAR ALCOHOL AND WATER.
Nickel-chromium oxide catalysts

Catalyst	Temp., °C.	Composition of gaseous products, % by vol.				
		CO_2	H_2	C_2H_4	CH_4	CO
52 80% Ni	240	8.3	40.8	1.4	30.2	19.5
	300	16.4	39.4	0.5	32.4	11.3
	350	22.0	31.8	0.5	40.9	4.7
	400	21.6	32.2	0.5	40.0	4.8
	450	20.4	40.4	0.4	32.1	6.8
	500	16.0	49.3	0.4	23.9	10.4
56 97% Ni	235	16.6	39.0	0.7	33.9	9.9
	265	23.5	25.4	0.8	48.1	2.3
	300	24.4	17.8	0.4	57.0	0.6
	325	24.3	29.0	0.6	43.8	2.4
	350	23.1	30.9	0.6	43.1	2.2

denced by the much higher percentage of this compound at 450° C. Such results are in accord with those to be discussed on the formation of carbon dioxide.

A series of catalysts were prepared from nickel and chromium and the results obtained with two of these are presented in Table V. These data have been taken from a previous paper (8).

The results with the catalysts shown in Table V are very interesting. It will be noticed that at a certain temperature, varying with the catalyst, the carbon dioxide and methane reached a maximum, while the carbon monoxide and hydrogen reached a minimum. It seems from this that the extent of decomposition of carbon monoxide varies with the temperature in a regular manner. Consequently the percentage of carbon dioxide formed varies in the same manner, and an increase in the amount of carbon dioxide accelerates the hydrogenation reaction, involving decreases in the hydrogen content and an increase in the amount of methane. With this series of catalysts this type of reaction was observed to be the usual one. Most catalysts did not behave in so clear-cut a manner, however, as shown by the products obtained when the catalysts of Audibert and Raineau (4) were used. These catalysts contained, in the case of No. 44, iron, copper, manganese, phosphorus and potassium, while No. 45 contained iron, copper, manganese, boron and potassium. The results are given in Table VI. With results such as these the source of the methane is rather difficult to determine with any degree of certainty. No carbon was deposited on the catalyst.

TABLE VI

EQUIMOLAR ALCOHOL AND WATER.
Audibert and Raineau catalysts

Catalyst	Temp., ° C.	Composition of gaseous products, % by vol.				
		CO_2	H_2	CH_4	C_2H_4	CO
44	350	6.9	89.3	0.7	3.1	Nil
	400	18.5	78.3	Nil	3.2	Nil
45	350	7.3	83.6	8.0	1.2	Nil
	400	18.2	77.9	1.5	2.5	Nil

The Formation of Ethane

A large number of catalysts have been investigated in this research, and some of the results have been discussed in another paper (7). It might be expected that a lower space velocity would increase the possibility of hydrogenation of ethylene when a catalyst that possesses the properties necessary to promote this reaction is used. Actually the results have indicated that this is the case with certain catalysts which cause ethane formation in the pyrolysis of carbon dioxide and alcohol mixtures but not in the pyrolysis of alcohol and water. The space velocity of the alcohol was much less when carbon dioxide was added. This, however, does not preclude the possibility of the hydrogenation reaction occurring at higher space velocities.

On the other hand, where the source of the ethane is the primary reaction of Bischoff and Adkins, it might be expected that the presence of diluents

such as carbon dioxide or water would serve merely to reduce the proportional quantity of ethane formed in the first case; or reduce the catalytic area available for alcohol decomposition, with the subsequent reduction of ethane production in the second case. These effects have been identified quite definitely with some catalysts.

Indications have also been obtained of the heat sensitivity of both of these reactions as shown by the change in activity of the catalyst with various treatments, such as oxidation and reduction between experiments. These conclusions are clearly illustrated in the results.

A variety of catalysts have been used and their preparation will be briefly described, except those already given. The numbers refer to the series used in this laboratory and are retained in this form for convenience in further references.

Catalyst No. 16—An equimolar mixture of zinc oxide and chromic oxide triturated in water and dried at 110° C.

Catalyst No. 28C—Similar to 28A but containing nickel as well as copper and chromium.

Catalyst No. 30—Ten moles of magnesium and one mole of chromium precipitated together, using ammonium hydroxide and a solution of the nitrates.

Catalyst No. 31B—One mole of cupric hydroxide from cupric chloride by precipitation with sodium hydroxide, with the addition of one-tenth mole of manganese dioxide as a promoter.

Catalyst No. 31F—As with 31B but using $\frac{1}{20}$ mole of each of titanic and molybdic acids as promoters.

Catalyst No. 33C—75% of nickel and 25% of zinc as hydroxides, by precipitation from a nitrate solution with sodium hydroxide.

Catalyst No. 36A—One mole of precipitated chromic hydroxide containing $\frac{1}{10}$ mole of titanic acid.

Catalyst No. 36B—As 36A, with lead oxide as the promoter.

Catalyst No. 36C—As 36A, with molybdic acid as the promoter.

Catalyst No. 41—Five moles of zinc oxide and one mole of molybdic oxide suspended in water. The suspension was evaporated to dryness with constant stirring using a motor stirrer.

The results given in Table VII are those obtained with carbon dioxide and alcohol. The column "Space velocity" refers to the rate of flow of carbon dioxide in cc. per min.; with those having a value of 25 cc. per min. the rate of alcohol flow was about 0.04 cc. per min. Table VIII contains results of experiments in which alcohol and water were used with rates of flow that varied slightly, as indicated. A comparison of results obtained with certain catalysts when carbon dioxide and water were used as the diluents, with the consequent variation in space velocity, is shown in Table IX. The results with catalyst No. 33C, and using carbon dioxide or water and alcohol and absolute alcohol, are given in Table X where a ready comparison is possible.

TABLE VII

RESULTS OBTAINED WITH CARBON DIOXIDE AND ALCOHOL

Catalyst No.	Temp., ° C.	Composition of gaseous products, % by vol.			Space velocity
		C_2H_4	H_2	C_2H_6	
16	450	2.5	35.6	4.3	50
	450	2.9	35.3	2.7	25
24	350	0.6	14.6	5.5	50
	450	1.8	36.5	9.1	30
	450	2.6	33.3	2.3	50
30	350	0.2	2.3	3.0	25
	450	1.6	27.4	8.7	25
31B	450	1.0	36.1	8.6	25
	450	0.4	11.0	2.4	100
31F	450	0.6	25.6	7.7	25
33C	450	Nil	17.9	10.7	25
	350	Nil	12.6	Nil	25
		Oxidized and reduced			
	300	Nil	29.0	6.3	25
	275	Nil	27.8	Nil	25
41	350	3.1	16.1	5.5	30

An examination of the results in Table VII points quite definitely to hydrogenation of ethylene as the source of ethane in every case except Catalyst No. 33C. With catalysts Nos. 24 and 30 the temperature effect is quite evident, the total reaction not being as great at 350° C. In consequence of the reactants available for hydrogenation being diminished, the ethane content is correspondingly lower. Catalysts Nos. 24 and 31B illustrate the influence of space velocity; the higher the velocity the lower the ethane content as might be expected from the decreased time the reactants are in contact with the catalyst. In the case of catalyst No. 33C it does not seem probable that any ethylene produced would be completely hydrogenated but indicates rather

TABLE VIII

RESULTS OBTAINED WITH WATER AND ALCOHOL

Catalyst No.	Temp., ° C.	Composition of gaseous products, % by vol.						Rate of flow, cc./min.
		CO_2	C_2H_4	H_2	CO	CH_4	C_2H_6	
28B	450	10.3	21.9	61.0	0.6	5.3	0.9	0.20
28C	350	1.1	56.8	42.1	Nil	Nil	Nil	0.27
	400	3.4	57.7	35.1	Nil	3.8	Nil	0.25
	450	9.2	45.0	36.2	1.1	8.5	Nil	0.31
33C	300	3.0	0.4	65.0	10.0	15.0	6.6	0.35
	500	20.4	Nil	44.6	6.8	22.3	5.9	0.33
			Oxidized and reduced					
	275	2.5	0.2	56.5	15.3	23.6	1.9	0.37
36A	450	2.3	38.5	49.7	Nil	3.0	6.5	0.55
36B	450	9.7	17.9	8.2	0.2	Nil	64.0	0.53
36C	450	5.8	21.0	69.0	Nil	Nil	4.2	0.60

TABLE IX

COMPARISON OF RESULTS OBTAINED WITH WATER AND CARBON DIOXIDE

Catalyst No.	Temp., °C.	Alcohol with	Composition of gaseous products, % by vol.			Rate of flow, cc./min.
			C_2H_4	H_2	C_2H_6	
24	450	H_2O	3.5	89.2	Nil	0.21
	500		3.5	86.2	Nil	0.21
	350	CO_2	0.6	14.6	5.5	0.08
	450		2.6	33.3	2.3	0.08
	450		1.8	36.5	9.1	0.08
30	450	Alone	14.0	80.9	Nil	0.55
	500		12.7	71.3	Nil	0.55
	350	CO_2	0.2	2.3	3.0	0.04
	450		1.6	27.4	8.7	0.04
41	350	H_2O	12.7	56.3	Nil	0.40
	450		10.8	69.5	Nil	0.63
	350	CO_2	3.1	16.1	5.5	0.04

TABLE X

COMPARISON OF RESULTS OBTAINED WITH CATALYST No. 33C

Alcohol with	Temp., °C.	Composition of gaseous products, % by vol.			Rate of flow, cc./min.
		C_2H_4	H_2	C_2H_6	
CO_2	350	Nil	12.6	Nil	0.04
	450	Nil	17.9	10.7	0.04
	Oxidized and reduced				
	275	Nil	27.8	Nil	0.05
	300	Nil	29.0	6.3	0.05
H_2O	300	0.4	65.0	6.7	0.35
	500	Nil	44.6	5.9	0.33
	Oxidized and reduced				
	275	0.2	56.5	1.9	0.37
Alone	300	1.2	59.8	11.2	0.23
	Oxidized and reduced				
	375	3.2	50.7	13.6	0.24
	450	3.4	53.0	17.1	0.31

that ethylene was not produced. This assumption seems justified as will be shown later. The temperature effect is quite evident with this catalyst and the variation in activity with vigorous treatment is also shown by the change in reaction following oxidation and reduction. This action of the catalyst is more evident in Table X.

The experimental results shown in Table VIII are more variant in nature. Catalyst No. 28A has been reported previously (7) and from a consideration of the results with catalyst No. 28B it would seem that the only effect of the addition of alkali to the catalyst was to reduce its activity and possibly promote polymerization of ethylene. If the ethane produced with these silica catalysts had come from the hydrogenation of ethylene, it was anticipated that the addition of nickel would increase this hydrogenation reaction. However as shown by the results with catalyst No. 28C there was no ethane

formed. The primary reaction was apparently inhibited by the presence of nickel in the catalyst and no indication of hydrogenation was evident. This result confirms the primary mechanism as the source of ethane with catalysts Nos. 28A and 28B. The results with catalyst No. 33C will be discussed later but the small ethylene content renders the hydrogenation reaction improbable in view of the results obtained with carbon dioxide and alcohol.

The dehydrating action of chromium is well known and the results obtained with catalyst No. 36 were therefore not unexpected. From the results shown in Table VII with catalyst No. 31F containing titanium and molybdenum it may be said that these metals behave similarly to chromium, promoting hydrogenation of ethylene. This action is quite evident with catalysts Nos. 36A and 36C. The catalytic activity of lead oxide has not been thoroughly examined, but Madenwald, Henke and Brown (27) found that it was very active in the hydrogenation of nitrobenzene and consequently the high percentage of ethane with catalyst No. 36B can be readily accounted for on the basis of this activity. In these chromium catalysts the source of ethane is quite evidently hydrogenation of ethylene.

In Table IX the results obtained with three catalysts, using carbon dioxide and alcohol, and water and alcohol, are compared. The conclusions which may be drawn are quite evident. In each case the decreased rate of alcohol flow using carbon dioxide is accompanied by ethane formation, due undoubtedly to hydrogenation of ethylene.

The primary formation of ethane with catalyst No. 33C is clearly shown in Table X. As already mentioned the presence of diluents serves only to decrease the volume of alcohol decomposed and decrease the space velocity. With carbon dioxide and alcohol the actual percentage of ethane produced in the decomposition is much greater than that indicated by the gas analysis, owing to the presence of excess carbon dioxide as carrier. In the case of diluted alcohol the presence of water reduces the amount of alcohol which will come in contact with the catalyst, and the extent of reaction is decreased. Using absolute alcohol, however, both of these effects are overcome, and this is clearly shown in the values obtained for ethane.

The Formation of Carbon Dioxide

The results to be presented are not entirely in agreement with the mechanisms already discussed. Several experiments showed the production of carbon dioxide under conditions that preclude the decomposition of carbon monoxide. The results of these experiments were of two types: (i) in some cases a gas, largely carbon dioxide and hydrogen, and oily condensates were obtained; (ii) in others, a gas was obtained as in (i), but there was no oily condensate. The mechanisms proposed by Lazier and Adkins and by Brown and Reid, previously described, might account for the first of these classes. An objection lies in the small yields of higher hydrocarbons found and the absence of propane. As a result, and to cover the second class, a new mechanism is proposed. It is not necessarily exclusive but rather is supplementary

to the mechanism of Lazier and Adkins. Confirmation of the possibility of the ketene reaction has been obtained and a hydrocarbon oil of the empirical formula of ethylene, but of high molecular weight, has been obtained. In addition oils were obtained repeatedly of the empirical formula C_5H_8O, approximately. The boiling point and molecular weight were high.

Table XI shows the results obtained with catalyst No. 33B and should be considered together with Table I. Catalyst No. 33B was made by precipitating zinc and nickel oxides from an equimolar solution of the nitrates, washing the precipitate, drying at 110° C., and reducing with hydrogen at 300° C. It is quite evident from the data that the origin of the carbon dioxide can be explained simply as due to the decomposition of carbon monoxide produced from acetaldehyde formed in the primary reaction. The parallelism between total oxides of carbon and methane is definite evidence.

TABLE XI

EQUIMOLAR ALCOHOL AND WATER OVER CATALYST No. 33B

Temp., ° C.	Composition of gaseous products, % by vol.			
	CO_2	CO	CH_4	Total oxides of carbon, as CO
275	1.8	4.4	11.5	8.0
325	2.7	5.3	10.7	10.7
450	4.0	4.6	13.0	12.6

The copper-chromium-silica catalyst previously discussed (5) produced very little carbon monoxide and no free carbon, but did yield a considerable amount of carbon dioxide.

Very little oil was produced and evidently other reactions yielding carbon dioxide were taking place to a considerable extent. The behavior of this catalyst and those of a similar nature will be referred to later.

As already mentioned, two types of oils have been identified; one a pure hydrocarbon and the other an oxygenated compound. As a catalyst capable of producing the former substance No. 30 was typical. On passing a mixture of carbon dioxide and alcohol vapor over this catalyst, a small amount of oil was obtained which showed on combustion analysis, 13% of hydrogen and 85.9% of carbon, corresponding approximately to an empirical formula of C_2H_4. It had a high molecular weight and a high boiling point. Such a substance might be formed from a ketene according to Adkins or by the polymerization of ethylene; the low percentage of ethylene in the gas coupled with the fact that alumina is primarily dehydrating (42) renders the latter reaction probable. Decarboxylation of an ester does not appear as reasonable.

The oxygenated compound has been identified in several cases using alcohol and water mixtures, and examples of the gas analyses obtained from reactions of this nature are given in Table XII. The results with catalyst No. 30, given in Table IX and repeated here for two temperatures, were obtained with absolute alcohol.

In all of these cases the oil closely approximated a composition corresponding to the formula C_6H_8O, and appeared to be a polymer of high molecular weight. The oil obtained with catalyst No. 30 was tested by the cryoscopic method for its molecular weight using benzene as the solvent, and a value of 240 was obtained, which probably corresponds to a trimer. The analyses of the off-gases show relatively low amounts of carbon monoxide and methane, which indicates slight secondary decomposition of acetaldehyde. The large hydrogen content, on the other hand, indicates considerable dehydrogenation, and these facts seem to conform with a mechanism to be suggested, in which much of the aldehyde is assumed to undergo further reaction. The water formed in the dehydration of the alcohol, or originally present in the mixture, might have reacted to produce carbon dioxide, but the absence of other products of these reactions reduces such a possibility.

TABLE XII

EXPERIMENTS GIVING CARBON DIOXIDE AND HYDROGEN AND OIL

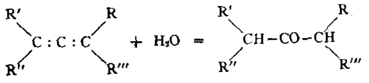
Catalyst No.	Temp., °C.	Composition of gaseous products, % by vol.				
		CO_2	C_2H_4	H_2	CO	CH_4
22	400	9.2	2.3	88.6	Nil	Nil
	450	8.7	4.9	85.5	Nil	1.0
	500	14.7	3.8	77.8	Nil	3.6
24	450	8.3	3.5	89.2	Nil	Nil
	500	10.6	3.5	86.0	Nil	Nil
27	350	7.3	1.6	91.0	Nil	Nil
	400	18.5	2.8	78.5	Nil	Nil
	450	18.9	3.1	76.8	0.7	0.7
	500	18.9	4.3	76.7	Nil	Nil
30	450	4.8	14.0	80.9	Nil	Nil
	500	10.4	12.7	71.3	2.6	2.9

The following mechanism is offered to account for these oils. This mechanism involves preliminary decomposition of the ketene with the formation of allene and carbon dioxide.

14. $2H_2CCO = CO_2 + H_2CCCH_2$.

Allene readily undergoes various reactions owing to its chemical activity. Lebedev (23) has reported the ease with which it is polymerized, forming compounds from the dimer to the hexamer. He also noted that the trimer was readily oxidized. Meinert and Hurd (30) also found polymerization of allene at 400–600° C. to proceed relatively easily. He obtained 90% conversion to the liquid dimer and trimer at 500° C. Allene hydrocarbons readily add on water in the presence of catalysts to form ketones (42).

15.



In this case acetone would be formed, and Adkins (42) states that in the presence of certain catalysts acetone will condense.

However, an addition reaction between allene and acetaldehyde would yield directly a compound with the composition C_5H_8O. The exact chemical

nature of such a product is difficult to predict, but there are several compounds, such as ethylidene acetone or tiglic aldehyde, which could be formed by a condensation reaction. The compound, C_6H_8O, being unsaturated, would be likely to condense to a polymeric form. Such a polymer might possess the properties shown by the substance found, and apparently the experimental conditions favor its formation. The slight variations in the molecular percentages shown on combustion analyses may be explained by the presence of small amounts of other substances which are possible products of the reaction. Owing to the relatively small quantities of product obtainable, complete separation and identification are very difficult. The reaction previously outlined for the original ketene decomposition involving acid formation will still be possible. It is interesting to note that Losanitsch (26) found that the exposure of ethylene and carbon monoxide mixtures to a silent electric discharge resulted in the formation of a dark brown liquid. The liquid had the formula C_5H_8O and appeared to be a dimer. Such or similar reactions cannot be ruled out in the present case.

The chief objection to the mechanism proposed lies in Reaction 14. There is no evidence for this reaction and it has been shown (35) that ketene decomposes to carbon monoxide and ethylene. This is true for temperatures in excess of 1000° C. over platinum and does not rule out entirely the reaction proposed.

A third type of result is given in Table XIII using equimolar alcohol and water and the catalysts described below. The gas produced was essentially carbon dioxide and hydrogen, and the liquids contained no high boiling fractions at all. Carbon was not deposited in appreciable amounts on the catalysts. The catalysts were prepared as follows: No. 37 was normal zinc chromate, precipitated from zinc nitrate with potassium chromate. Catalyst No. 43 contained five moles of zinc hydroxide and one mole of chromic hydroxide precipitated from the nitrates with potassium hydroxide, and washed thoroughly. Catalysts Nos. 44 and 45, the same as used previously, were prepared by the method of Audibert and Raineau (4) and contained copper, manganese, iron, potassium and phosphorus or boron.

At first sight, reaction No. 3 or 5 or both together with dehydrogenation of alcohol to aldehyde might appear to account essenti-

TABLE XIII

EXPERIMENTS GIVING CARBON DIOXIDE AND HYDROGEN WITH NO OIL

Catalyst No.	Temp., ° C.	Composition of gaseous products, % by vol.				
		CO_2	C_2H_4	H_2	CO	CH_4
37	350	5.6	6.7	76.3	3.3	8.1
	450	17.3	2.4	77.7	1.3	1.1
43	350	5.3	4.0	88.6	Nil	2.1
	450	12.9	6.4	79.6	Nil	1.1
44	350	7.3	1.4	83.3	Nil	8.0
	450	18.1	2.5	77.7	Nil	1.6
45	350	6.8	3.0	88.1	0.2	1.9
	450	15.4	4.0	78.6	Nil	Nil

/

ally for these results. It is necessary only to assume some slight dehydration also to account for the ethylene. This may be true at 350° C., but is not adequate to explain the results at 450° C. The absence of carbon formation, the equilibrium conditions of reaction No. 3 or 5 and the small amounts of methane and carbon monoxide, compared to the production of carbon dioxide, taken together make it necessary to seek some other mechanism. No satisfactory mechanism can be proposed at present but the following may be suggested.

16. $C_2H_5OH + H_2O = 2CH_3OH$
17. $CH_3OH + H_2O = CO_2 + 3H_2$.

Three difficulties are at once apparent. The first reaction, 16, has not been shown to occur, although the isomer of ethyl alcohol, methyl ether, will behave in such a manner. Second, methyl alcohol was not found among the products of the reaction and its presence might be expected in small amounts at least. Lastly and most important, it is probable from work done here and elsewhere that the second reaction, 17, occurs in two stages; the decomposition of methanol to carbon monoxide and hydrogen followed by the water-gas reaction giving carbon dioxide. The small amounts of carbon monoxide surviving make the reaction extremely doubtful.

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NON-BASIC CONSTITUENTS OF THE LEAVES AND ROOTS OF *ADLUMIA FUNGOSA*, GREENE[1]

By Léo Marion[2]

Abstract

An investigation of the water-insoluble constituents of this plant has disclosed the presence of an unidentified substance, $C_{28}H_{56}O_2$(?), of adlumiasterol which forms a monoacetate, of a phenolic substance occurring with a mixture of fatty acids, and a red pigment, m.p. 286° C. Fumaric acid and a sterolin also occur together with 3 : 4–methylenedioxyphthalide which is obtainable as well by hydrolysis of adlumine.

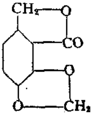
Small quantities of the four alkaloids protopine, adlumine, adlumidine and bicuculline were also present.

The present investigation is incidental to that carried out by Manske on the alkaloids of *Adlumia fungosa*, Greene (1). In the course of his work on this plant Manske obtained a methanolic extract E, which could be separated into a fraction S, soluble in dilute acid, and a residue R, insoluble in that medium. It is the study of that residue which is now reported.

Previous investigators of this plant have largely restricted their attention to its alkaloids and attached little or no importance to the non-basic constituents (4, 5). Besides the four bases, protopine, adlumine, adlumidine and bicuculline, small quantities of which were still present in the water-insoluble fraction, the latter contained a substance, $C_{28}H_{56}O_2$, m.p. 82° C., which suffers no depression in melting point when admixed with cerotic acid, but does not possess the properties of either an acid or an ester. When treated with various reagents it fails to produce the usual derivatives of an alcohol. A sterol, $C_{39}H_{68}O_2$, is also present in the water-insoluble fraction; it belongs to the type $C_nH_{2n-10}O_2$, examples of which are on record (3). As in the cases already known it gives rise to a monoacetate only. For convenience it may be designated adlumiasterol, from the generic name of the plant. In the accumulated mother liquors of adlumiasterol a red pigment, m.p. 286° C., was found to occur in very small quantity.

The fat, separated with the aid of petroleum ether, yielded a mixture of fatty acids and a substance, insoluble in sodium bicarbonate but soluble in sodium hydroxide, which possesses phenolic properties and melts at 115° C.

A large quantity of fumaric acid had already been isolated from the water-soluble extract and a small amount of the same acid was found present in the insoluble residue, together with a substance identified as 3:4–methylenedioxyphthalide (I) derivable from adlumine. Whether 3:4–methylenedioxyphthalide occurs as such in the plant cannot be definitely stated, since its production by hydrolysis of adlumine in the course of the process of isolation of the alkaloids appears equally possible.



(I)

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The extract also contained an appreciable quantity of a sterolin, $C_{33}H_{56}O_6$, which was characterized by the preparation of its acetate and benzoate; on hydrolysis, it produced a sterol and glucose. In this connection it is noteworthy that the sterol obtained on hydrolysis of the sterolin is different from that which occurs in the free condition in the plant.

Experimental

The dried and ground material (11 kilos) when thoroughly extracted with methanol yielded a solution (extract E) which was separated into an acid-soluble fraction S and an insoluble residue R (1). Besides containing much chlorophyll, the residue was very soft and oily; in order to facilitate its extraction in a Soxhlet apparatus, it was found expedient to mix it with shredded asbestos. This was done with the aid of alcohol which was subsequently evaporated. The hard cake thus produced was broken up and extracted successively with petroleum ether, ether, chloroform, ethyl acetate and methanol.

Petroleum Ether Extract

Following the removal of the solvent by distillation the residual oil was saponified with alcoholic potash and separated into acidic and unsaponifiable fractions.

Isolation of a Substance, $C_{28}H_{56}O_2$(?)

A solution of the unsaponifiable matter in hot acetone deposited, on cooling, copious shining flakes of a substance (9.3 gm.) which, after several recrystallizations from boiling acetone, melted at 81–82° C. When distilled under diminished pressure (255–256°/1.5 mm.) and recrystallized again from boiling acetone, this substance melts at 82–83° C. Calcd. for $C_{28}H_{56}O_2$: C, 79.24; H, 13.21%; M.W. 424. Found: C, 79.41, 79.54; H, 13.78, 13.72%; M.W. 413, 404 (Rast). It could not be saponified, and an attempt to esterify it by boiling with methanolic sulphuric acid likewise yielded the unchanged substance. Treatment with acetic anhydride, *p*-nitrobenzoyl chloride and phenylisocyanate failed to produce derivatives. Owing to the facts that the composition of the substance is very close to that of cerotic acid, that it has the same melting point and that admixture with an authentic specimen did not depress the melting point, the following experiment was carried out to prove, or disprove, the possible identity. A solution of a known weight of the substance in hot alcohol was titrated in the presence of phenolphthalein against standardized base. It did not neutralize any base whereas cerotic acid under the same conditions can be titrated readily. An attempt to oxidize the substance with a solution of chromic acid in glacial acetic acid produced an acid which, recrystallized from methanol, melted at 59° C. Although the substance appears to be an alcohol, its abnormal behavior is such as to make its identity extremely uncertain.

Isolation of Adlumiasterol, $C_{30}H_{48}O_2$

The acetone solution from which the aliphatic alcohol had crystallized was distilled and the crystalline residue dissolved in boiling methanol.

Repeated separation from boiling methanol finally yielded the sterol as lustrous flakes melting at 151–152° C. Calcd. for $C_{39}H_{68}O_2$: C, 82.39; H, 11.98%. Found: C, 82.72, 82.47; H, 11.74, 11.83%. The acetate, prepared by boiling with acetic anhydride in the presence of pyridine, melts at 136–137° C. Calcd. for $C_{41}H_{70}O_3$: C, 80.6; H, 11.48%. Found: C, 81.93; H, 11.16%. Adlumiasterol, as this substance might be named, therefore, belongs to a class of the general formula, $C_nH_{2n-10}O_2$, the known examples of which, however, are not identical with it.

The accumulated mother liquors from the recrystallizations of adlumiasterol exhibited a deep red coloration and, on concentration, yielded a dark red oil. The oil was dissolved in a little hot methanol (charcoal) and the solution allowed to evaporate spontaneously. After prolonged standing the residue crystallized into a mixture of flakes and slender prisms of a beautiful deep red color. The latter were separated mechanically, freed of adhering sterol by rapid washing with warm methanol and dried, m.p. 286° C. The quantity of crystalline pigment which could be freed of sterol and isolated in an uncontaminated condition was, unfortunately, too small for analysis.

Isolation of a Phenolic Substance

The ethereal solution of the liberated acids obtained from the saponified fat was extracted with a 5% aqueous solution of sodium bicarbonate which removed the fatty acids, and with a 5% aqueous solution of sodium hydroxide. A stream of carbon dioxide was passed through the latter extract and the precipitated substance was collected in ether and recrystallized several times from a mixture of ethyl acetate and methanol. It separated from this solvent as small, white crystals melting at 115° C. Calcd. for $C_8H_{16}O$: C, 75.0; H, 12.49%. Found: C, 75.04, 75.45; H, 12.33, 12.44%. The quantity isolated was too small to permit further identification. The fatty acids were not further investigated.

Ether Extract

The residue obtained on distillation of the ether consisted of a dark green, resinous mass mixed with a white, crystalline material. It was digested with one litre of ether and filtered in order to separate the white material which was less soluble than the rest.

Isolation of Fumaric Acid

The ethereal solution was shaken with 5% aqueous solutions of ammonium carbonate, potassium carbonate and sodium hydroxide, and each of these solutions was acidified and extracted with ether. In the course of the extraction with potassium carbonate, a crystalline substance separated out at the interface; it was collected and treated as described further on. From the ammonium carbonate solution was obtained an oil which could not be crystallized by separation from various solvents and was therefore boiled with methanolic sulphuric acid. Fractionation of the esterified product yielded a substance, b.p. about 65° C./2.5–3 mm., which crystallized in the condenser. This melted at 98–99° C. and when mixed with an authentic specimen of

methyl fumarate, m.p. 100–101° C., caused no depression in melting point. Small amounts of higher boiling fractions were also obtained, but in too small a quantity to permit identification.

Isolation of 3:4–methylenedioxyphthalide

From the potassium carbonate solution a mixture of oily substances was obtained which was boiled with methanolic sulphuric acid. This seemed to remove an oily impurity and the product on distillation yielded an oil, b.p. 164–5° C./3.5 mm., which on standing deposited a very small quantity of acicular colorless crystals which, recrystallized from methanol (charcoal) and from ether, melted at 225–226° C. The oil from which the above substance had separated crystallized when cooled, but melted again at room temperature. It apparently consisted of a mixture of methyl oleate and stearate (b.p. 150–152° C./3 mm.). The residue left in the distilling flask was dissolved in methanol (charcoal) and the solution allowed to stand, when it slowly deposited a further quantity of the same crystalline substance as above, melting at 225° C.

The sodium hydroxide extract yielded a dark green oil which was easily dissolved in methanol and boiled with sulphuric acid. The oily product was dissolved in ether and the solution washed with sodium bicarbonate and water, dried and evaporated. It yielded an oil which separated on cooling from solution in a mixture of ethyl acetate-methanol as long, colorless needles, m.p. 229–230° C. This substance, which is the same as that obtained from the potassium carbonate solution, is identical with 3:4–methylenedioxyphthalide isolated by Manske (1) from one of the products of the hydrolytic oxidation of the alkaloid adlumine. Admixed with a small quantity of the latter it began to sinter at 229° and melted completely at 232–233° C. The melt had crystallized again on cooling at 226° C.

Isolation of a Phytosterolin

That part of the original ether extract which was sparingly soluble in that solvent was digested with hot dioxane and the filtered solution allowed to cool. A white, crystalline substance gradually separated which was recrystallized from a mixture of dioxane and alcohol (charcoal), m.p. 295° C. Calcd. for $C_{33}H_{56}O_6$: C, 72.25; H, 10.21%. Found: C, 72.33, 72.17; H, 10.12, 10.24%. The interaction of benzoyl chloride with the substance in pyridine solution forms a tetrabenzoate, m.p. 184–5° C. (calcd. for $C_{33}H_{52}O_6(CO.C_6H_5)_4$: C, 75.9; H, 7.5%; found: C, 76.26; H, 7.74%), and boiling with acetic anhydride containing a small amount of pyridine yields a tetracetate, m.p. 157° C. Calcd. for $C_{33}H_{52}O_6(COCH_3)_4$: C, 68.7; H, 8.9%. Found: C, 69.31; H, 9.11%. The compound, which gave positive color tests, is undoubtedly a phytosterolin. Inasmuch as adlumiasterol is the only sterol found in the unsaponifiable fraction it was interesting to determine what sterol formed a constituent part of the sterolin. To this end 0.2 gm. of the sterolin was hydrolyzed in amyl alcoholic solution with aqueous hydrochloric acid according to Power and Salway's method (2). The amyl alcohol was

removed in a current of steam and the precipitated sterol filtered and recrystallized from a boiling mixture of methanol and ethyl acetate (charcoal) from which it separated in pearly flakes, m.p. 135–136° C. Admixed with the sterol obtained by hydrolysis of a sterolin isolated from the seeds of *Asclepias syriaca*, it melted at 135° C. Hence, the sterol constituent of the sterolin is quite different from that occurring in the free condition. The aqueous filtrate on treatment with phenylhydrazine yielded phenylglucosozone, m.p. 205° C.

Isolation of Adlumine and Adlumidine

The dioxane-alcohol mother liquors from the first crystallization of the phytosterolin, when further concentrated, yielded orange-colored prisms which, recrystallized from a mixture of acetone and methanol, separated in light yellow prisms, m.p. 237° C., was identical with adlumidine, $C_{19}H_{17}O_6N$. Admixture with an authentic specimen failed to cause any depression in melting point.

In the course of the extraction of the ether solution with 5% aqueous potassium carbonate as already mentioned, a white substance separated as glistening flakes. This, when recrystallized from dilute alcohol, yielded long slender needles which, after several recrystallizations, melted at 179° C. It was identical with adlumine and when admixed with an authentic specimen still melted at 179° C.

Chloroform Extract

The chloroform extract was filtered and shaken successively with 5% aqueous solutions of ammonium carbonate, sodium bicarbonate and sodium hydroxide. None of these aqueous extracts, however, yielded anything crystalline.

Isolation of Protopine and Bicuculline

The chloroform solution was then reduced to about 250 cc. and poured into an equal volume of ether which caused the separation of some gummy material. This was filtered out and the purplish fluorescent filtrate deprived of solvent, the oily residue dissolved in boiling methanol (charcoal) and the solution allowed to cool. The yellow crystalline material which was deposited was recrystallized from acetone-methanol and found to consist of adlumidine, m.p. 237° C. The methanol mother liquor on further standing deposited a mixture of very small crystals, large truncated octahedra and stellate aggregates of prismatic needles, which were separated mechanically and recrystallized. The respective substances were found to consist of the alkaloids adlumidine, adlumine, m.p. 180° C., and protopine $C_{20}H_{19}O_5N$, m.p. 209° C.

From the accumulated mother liquors a residue was obtained which yielded the alkaloid bicuculline, $C_{20}H_{17}O_6N$, m.p. 176–177° C. Hence, no non-basic substances were present in the chloroform extract.

Ethyl Acetate and Methanol Extracts

These extracts yielded only non-crystallizable solids resembling lignin. They were boiled with 10% aqueous sodium hydroxide, acidified and extracted with ether but traces of oil only were obtained.

Acknowledgment

The author wishes to express his thanks to Dr. R. H. F. Manske, who supplied him with the raw material, and his appreciation for his helpful suggestions and interest.

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THE ALKALOIDS OF FUMARIACEOUS PLANTS

X. *DICENTRA OREGANA*, Eastwood[1]

By R. H. F. Manske[2]

Abstract

The examination of the alkaloids of *D. oregana* has yielded seven bases, one of which appears to be identical with *alkaloid* δ previously found in two other *Dicentra* spp. A second base has not been adequately characterized because of paucity of material and is referred to as *alkaloid* ε. The remaining five alkaloids which are well known are *dicentrine, glaucine, corydine, protopine*, and *α-allo-cryptopine*. The isolation of the last from a species of *Dicentra* is the first on record. Attention is called to the ratio of glaucine to dicentrine and its significance is discussed. The total yield of purified alkaloids was unusually high (over 3.0%).

The species of *Dicentra* under consideration was first described by Miss Alice Eastwood in the Proceedings of the California Academy of Science (1931, vol. 20, p. 144) and has not yet been listed in the Kew Index. It is therefore pertinent to quote in part from the above mentioned record.

"Perennial from thick, branching, scaly rootstocks; leaves glabrous and glaucous, ternately compound with the divisions pinnately dissected, ultimate segments confluent, laciniately dentate, blade 6–10 cm. long and broad, petioles 1–2 dm. long, dilated at base; scapes naked, 2–3 dm. high, striate; inflorescence terminal, nodding paniculate with the flowers closely clustered on filiform pedicels, bracts and bractlets filiform to linear-attenuate; sepals oblong-lanceolate, acuminate, striate, 6 mm. long, 2.5 mm. wide; corolla ovate-cordate, exterior petals ochroleucous with short spreading tips, inner with the exserted limb rose color; ovary smooth, shorter than the style."

"——— In the U.S. National Herbarium it is represented by a specimen collected by Thomas Howell at Waldo, Oregon, June 4, 1884, No. 3424. ———. Not only in the color of the flowers but in the pallid foliage, this species presents a quite different appearance of any of the numerous forms of the variable Dicentra formosa".

The material for the present investigation was collected with obvious care by a competent collector in Oregon. Nevertheless it was deemed necessary to have it properly authenticated and for this service the writer expresses his thanks to Dr. H. T. Güssow, Dominion Botanist, who in turn submitted it to Miss Alice Eastwood, Curator of the Herbarium of the California Academy of Sciences.

Chemically, *D. oregana* was found to conform to its botanical relationship with *D. formosa* (5) and *D. eximia* (4). Protopine, because of its ubiquitous occurrence in plants of the Fumariaceae family cannot be regarded as pointing to closer affinities within a genus. However, dicentrine, corydine and

[1] *Manuscript received April 19, 1934.*
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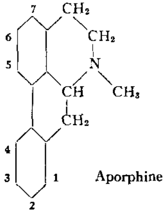
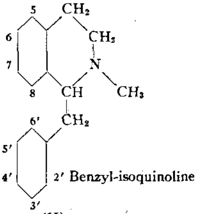
glaucine, particularly when all three occur in the one plant, indicate a chemical relation which in the three cases quoted is closely paralleled by botanical classification. The specificity of these three plants is nevertheless confirmed not only by the glaucine-dicentrine ratio (5), but by the appearance in each plant of one or more subsidiary alkaloids not common to the others.

Though it has not been possible to obtain an accurate assay of both glaucine and dicentrine, the respective yields serve as a fair approximation. On this basis the ratio of glaucine to dicentrine is 100 : 64, and this in comparison with the corresponding ratios for *D. eximia* (100 : 1300) and *D. formosa* (100 : 70) points to an enhanced tendency to methylation as opposed to methylenation.

A second point of phytochemical interest emerges from an inspection of the nature of the aporphine bases. In the genus *Corydalis* these bases are derived from 3 : 4 : 5 : 6-tetrahydroxy-aporphine by partial methylation or methylenation or both. There are two obscure exceptions, namely, the occurrence of *d*-glaucine (2 : 3 : 5 : 6-tetramethoxy-aporphine)



(I)



(II)

in the aerial portions of *C. cava* (1) and of *l*-glaucine in *C. ternata* (2). In the three *Dicentra* spp. under discussion however, the two types of aporphine bases (3 : 4 : 5 : 6- and 2 : 3 : 5 : 6-) occur side by side in quantities of the same order. Since the aporphine bases are probably derived from the 3′ : 4′ : 6 : 7-tetrahydroxy-benzyl-isoquinoline bases by oxidative ring closure at the 2′ : 8- or 6′ : 8- positions, the balance between the two types is certain to be a delicate one and slight changes of condition may have relatively large effects. Nevertheless, the catastrophic disappearance of one type or the other is difficult to conceive. The same is true of the ratio of methylation to methylenation products and the presence of allocryptopine (ratio to protopine, 100 : 210) in *D. oregana* is further evidence that the strong inclination to the former process is not confined to the aporphine bases, and that the specificity of this plant is assured. An abortive search for minute traces of alkaloids in the relatively small amounts of plant material available for these investigations cannot always affirm the complete absence of a particular base. With the proviso, however, that the absence of an alkaloid can be taken for granted

absent from *D. formosa*. On this basis corytuberine is not present in *D. oregana*, and its place is taken by an alkaloid which thus far has not been identified with any hitherto described. It is phenolic, probably contains two methoxyl groups, and yields analytical figures in fair agreement with $C_{19}H_{21}O_4N$ or with $C_{19}H_{23}O_4N$. Owing to the small amount available it will be referred to as alkaloid ϵ until adequate characterization is possible.

A seventh alkaloid was obtained in small amounts, and as far as identification has been possible it is regarded as identical with alkaloid δ first found in *D. eximia* and subsequently in *D. formosa*.

Experimental

There was available a total of 1515 gm. of dried plant material of which the roots constituted 1025 gm. The aerial portion and the roots were examined separately, but differences sufficient to place on record were not observed. The total yield of bases from the roots was however, slightly higher. The following record refers therefore only to the roots, and the designations of the various fractions are those of a previous communication (3).

Isolation of Dicentrine

Owing to the sparing solubility of the hydrochloride of this base it occurs not only in the fraction (BC), but the hot aqueous filtrate (S) and the aqueous filtrate (SR) frequently deposit the hydrochloride on cooling. In the present case these crops of hydrochloride were worked up separately. The fraction (BC) was again converted to hydrochloride and a further crop of dicentrine hydrochloride obtained in this way. The dicentrine was regenerated from this and the recrystallized base (m.p. 169° C.)* as well as the derivatives had the properties previously recorded (4). The yield of purified base was 0.76% which is appreciably higher than in any case on record.

Isolation of Glaucine

The free base was regenerated from the final dicentrine hydrochloride mother liquor by means of potassium hydroxide, gathered on a glass rod and thoroughly washed with water. The dried base was suspended in ether and the slight turbidity removed by filtration with the aid of charcoal. Evaporation to a thin syrup and addition of a crystal of *d*-glaucine induced immediate crystallization. After a convenient time the solid was filtered off, washed cautiously with ether (m.p. 117–118° C.) and recrystallized from boiling ether. Colorless crystals of *d*-glaucine were thus obtained, which alone, or admixed with specimens from *D. eximia* or *D. formosa*, melted at 120° C. It is difficult to obtain the last traces in a pure condition so that the yield of 1.19% is based on glaucine which was not of maximum purity.

Isolation of Corydine

The fraction (EC) was dissolved in methanol, treated with a slight excess of methanolic hydrogen chloride and the solution repeatedly evaporated with chloroform.

**Melting points are corrected.*

During the evaporation a sparingly soluble hydrochloride crystallized out. It was filtered off and washed with cold chloroform. The base was regenerated from this by treating a rapidly cooled aqueous solution with ammonia and extracting with ether. The ether extract on evaporation to a small volume deposited colorless triboluminescent prisms which melted at 120–122° C. either alone or admixed with a specimen of corydine similarly prepared from *D. eximia*. Although the rigorous identification which was recorded in the case of corydine from *D. formosa* was not repeated in this case, comparison with authentic specimens, and the properties of the hydrochloride, together with the general behavior, are considered sufficient. The yield was 0.05%.

Isolation of Alkaloid ε

This base was found in two fractions (EC) and (EEC). Had the extraction of the alkaline filtrate (FC) been more exhaustive it would probably not have been found in the latter fraction. This was dissolved in a mixture of chloroform and methanol, and the filtrate (charcoal) repeatedly evaporated with methanol. The somewhat concentrated solution deposited stout colorless prisms which melted at 220–222° C. with some previous sintering. After two further recrystallizations from chloroform-methanol the base melted at 230°, sintering beginning at 224–225° C.

It was also obtained from fraction (EC). For this purpose the chloroform mother liquor from the corydine hydrochloride was freed of solvent, the residue dissolved in water and the clarified filtrate basified with excess potassium hydroxide. The resulting turbidity was removed by filtration and the filtrate saturated with ammonium chloride. Ether then removed a small amount of base which when recrystallized from methanol melted with previous sintering at 228° C. Admixture with the base from fraction (EEC) did not lower the melting point. The combined yield was 0.01%.

The micro-Zeisel determination of methoxyl gave figures rather too high for two such groups. On the other hand the results for the combined methoxyl- and methylimino- groups are in fair agreement with two of the former and one of the latter.

Calcd. for $C_{19}H_{21}O_4N$: C, 69.74; H, 6.40; N, 4.28; 2OMe + NMe as OMe, 28.34%. Found: C, 69.67, 69.87; H, 6.70, 6.79; N, 4.13, 4.28; OMe + NMe, 27.25%.

Examination of fraction (BCE) for corytuberine failed to show its presence (5).

Isolation of Protopine

The fraction (BS) was dissolved in chloroform and the filtered solution (charcoal) repeatedly evaporated with methanol. The hot solution was inoculated with a crystal of protopine and rapid crystallization induced by shaking. The protopine (m.p. 208° C.) was filtered off, washed with methanol, and the combined filtrates evaporated to a thin syrup. A second crop of

specimen was authenticated by color reactions and mixed melting point determination (211° C.).

Isolation of α-Allocryptopine (β-Homochelidonine)

The methanolic filtrate from the crystallization of the protopine was evaporated to a small volume, neutralized with methanolic hydrogen chloride, and inoculated with a crystal of protopine hydrochloride. The small amount of the latter, which crystallized in the course of several days was filtered off and the filtrate poured into water. The methanol was boiled off and the filtered and cooled solution basified with excess potassium hydroxide. The granular precipitate was filtered off, washed, dried, and dissolved in chloroform. The filtered solution was repeatedly evaporated with methanol, finally to a thin syrup. To the latter a large volume of dry ether was added and the small amount of amorphous residue removed with the aid of charcoal. The clear filtrate was evaporated to a syrup. In the course of several days a single crystal nucleus had appeared. The addition of a little methanol and rubbing with a glass rod induced the crystallization of a copious crop of alkaloid which melted not quite sharply at 157–158° C. Small amounts of protopine were still present, but rapid solution in boiling methanol left the greater portion undissolved and the base which crystallized from the filtrate melted at 159–160° C. A repetition of the process yielded the pure base melting at 160–161° C. A crystal dissolved in acetic acid and treated with concentrated sulphuric acid developed a rich reddish-violet color. The β-form was not obtained. Gaebel's test for the methylene-dioxy group was positive though not nearly as intense as a comparative test with protopine. The yield was 0.33%. Calcd. for $C_{21}H_{23}O_5N$: N, 3.80; OMe, 17.17%. Found: N, 3.82, 3.82; OMe, 15.57, 14.50%.

Isolation of Alkaloid δ

This alkaloid, which has thus far not been obtained crystalline in the free condition, was isolated as the hydrochloride from the fractions (BSE) and (EES) in the manner recorded previously (4, 5). The criteria of identity have been,—the properties of the hydrochloride (no change in melting point on admixture), color reactions with sulphuric acid, and induction of crystallization of one salt in supersaturated solution by another. No observation which would serve to distinguish one from the others has been made. The yield of hydrochloride (m.p. 236–237° C. dec.) was somewhat less than 0.01%.

Isolation of Fumaric Acid

With the one exception of the tubers of *D. canadensis*, all plants which have come under this program of chemical examination have yielded fumaric acid in varying amounts. The fraction (LC) in the present investigation yielded less than 0.01%, but nevertheless, there was sufficient for proper identification after it was repeatedly recrystallized from water. It melted either alone or admixed with an authentic specimen of fumaric acid at 287–

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THE PREPARATION AND QUALITATIVE IDENTIFICATION OF 2,5-DIMETHYLPYRROLE[1]

By C. F. H. Allen[2] and D. M. Young[3]

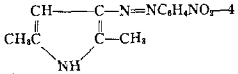
Abstract

A new and convenient method for preparing 2,5-dimethylpyrrole, by the interaction of acetonylacetone and ammonium carbonate, is described. It may be identified by the formation of three solid derivatives: (*a*) the azo compound; (*b*) the mono-2,4-dinitrophenylhydrazone of acetonylacetone; and (*c*) its condensation product with acetone.

The recent accessibility of acetonylacetone has made it possible to obtain hitherto uncommon substances in the heterocyclic series of five membered rings for investigation. Among these is 2,5-dimethylpyrrole. This substance has always been prepared from the diketone by the action of ammonia under pressure; the yields are about 50% (3, 6). By substituting ammonium carbonate, it is now readily obtained in any quantity in the usual laboratory apparatus.

The recognition of 2,5-dimethylpyrrole has been based on physical properties; the non-formation of characteristic solid derivatives is both unsatisfactory and inconvenient. If it reacts with halogen acids, picric acid, alkyl halides, and methyl *p*-toluene sulphonate, it does not give solid derivatives. It does not add maleic anhydride when mixtures are heated, either alone or in benzene.

Three reactions that are useful for identification have been found. First, the pyrrole couples with *p*-nitrodiazobenzene chloride. The azo compound



has been described previously (5). Second, it reacts very rapidly with 2,4-dinitrophenylhydrazine in the presence of dilute mineral acid, to give the mono-2,4-dinitrophenylhydrazone of acetonylacetone; this is essentially a reversal of the method of preparation of the pyrrole. The ease of opening of the pyrrole ring in this instance is noteworthy, but in agreement with the formation of the dioxime of acetonylacetone by the action of alkaline hydroxylamine on 2,5-dimethylpyrrole (1). There is very little in the literature on this and analogous reactions; prolonged boiling with alkaline hydroxylamine eventually yields small amounts of succindialdehyde dioxime with pyrrole, while 2,5-dimethylpyrrole and nitrous acid give the 2,3-dioxime of 1,4-dimethyltetraketone (4). No dinitrophenylhydrazone formation was noted with pyrrole, or 2,4-dimethyl-3,5-dicarbethoxypyrrole, even on long heating of the reactants; 2,4-dimethyl-3-acetyl-5-carbethoxypyrrole gave a dinitro-

[1] *Manuscript received April 11, 1934.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada.
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phenylhydrazone, presumably of the methyl ketone, since the ring nitrogen was still present. Third, in the presence of hydrochloric acid, 2,5-dimethylpyrrole and acetone form a crystalline substance of unknown structure, $C_{18}H_{26}N_2$ (6), when operations are performed in a particular manner.

Experimental

Acetonylacetone* (100 gm.) and 200 gm. of U.S.P. ammonium carbonate in lumps are placed in a 500 cc. Erlenmeyer flask, fitted with a large bore reflux condenser. The mixture is heated in an oil bath at 100° C. until effervescence stops (one hour) and refluxed a further half hour at 115° C. When cool, the upper pyrrole layer is separated and dried overnight with anhydrous calcium chloride in a stoppered flask, previously swept out with nitrogen. (From this point on, all manipulations must be done in an oxygen-free atmosphere.) It is then vacuum-distilled; the moist fore-runnings are collected separately, but the fraction boiling at 50–53° C. at 8 mm. (165–167° C. at 760 mm.) is retained. The average yield in a number of runs was 72 gm. or 87%. The product has a very slight yellowish tinge which gradually becomes red—this change is accelerated by light and air, so the pyrrole is best preserved under nitrogen in a brown glass container. The refractive index of the freshly distilled material is $1.5001_D^{22°}$; Nasini and Carrara give $1.5036_D^{19.8°}$ (2). Under the same conditions, 2,5-dimethylfurane does not react with ammonium carbonate, and is recovered unchanged.

Derivatives

(a) The azo compound. A solution of diazotized *p*-nitraniline is added to a cold mixture of 1 gm. of dimethylpyrrole in 5 cc. of acetic acid; the reddish-orange precipitate must be filtered at once to avoid decomposition. It is boiled a few minutes with 15–20 cc. of ether, filtered, washed with the same solvent and dried. It forms carmine needles that shrink suddenly at about 165° C. and melt to a liquid at about 200° C.; the analysis indicates a dihydrate. Calcd. for $C_{23}H_{12}O_2N_3 . 2H_2O$: N, 20.0%. Found: N, 20.1%. For purification it is dissolved in about 10 cc. of hot methyl alcohol and on addition of water, reddish-brown microscopic needles are precipitated. These crystals melt with decomposition at 208–212° C., according to the rate of heating. Since the melting point varies in this way, it is advisable to prepare an additional derivative, such as is described below. Three drops of the pyrrole is the minimum that can be detected by this procedure, with certainty.

(b) Mono-2,4-dinitrophenylhydrazone of acetonylacetone. A mixture of 0.5 gm. of 2,4-dinitrophenylhydrazine, 0.25 gm. of 2,5-dimethylpyrrole, and 25 cc. of 95% ethyl alcohol is heated to the boiling point and 1 cc. of 10% sulphuric acid added. An orange precipitate separates at once; it is filtered and recrystallized from pyridine, from which it separates in orange needles, m.p. 262° C. It is insoluble in the common organic solvents, except nitrobenzene, and in butyl alcohol, dioxane, cymene, 2,5-dimethylfurane, and amyl ether.

**The diketone used was supplied by Shawinigan Chemicals, Limited, and is gratefully acknowledged.*

Calcd. for $C_{12}H_{14}O_6N_4$: N, 19.0%. Found: N, 18.8%. It is identical with the derivative formed from acetonylacetone by the same procedure.

The 2,4-dinitrophenylhydrazone of 2,4-dimethyl-3-acetyl-5-carbethoxypyrrole was prepared in the same manner. It crystallizes in carmine rods from aniline, m.p. 264° C. Calcd. for $C_{17}H_{19}O_6N_5$: N, 18.0%. Found: N, 17.6%.

When pyrrole or 2,4-dimethyl-3,5-dicarbethoxypyrrole was treated by the same procedure, or much more drastically, the reagent and pyrrole ester were recovered unchanged; the unsubstituted pyrrole resinified.

(c) To a mixture of 2.5 cc. of 2,5-dimethylpyrrole and 8 cc. of acetone at room temperature are added four drops of concentrated hydrochloric acid. After five minutes the solution is poured into 20 cc. of water, the precipitate filtered, and recrystallized from 50% alcohol. It forms flesh-colored needles, m.p. 174° C., after two recrystallizations. Calcd. for $C_{18}H_{26}N_2$: N, 10.4%. Mol. wt. 270. Found: N, 10.9%. Mol. wt. 269. Slight variations lead to other products, but as little as 0.5 gm. of the pyrrole can be detected by the above procedure. The substance is not obtained when acetonylacetone is substituted for the pyrrole, nor when the acetone is replaced by diacetone-alcohol or mesityl oxide.

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THE RELATION BETWEEN YIELD AND PROTEIN CONTENT OF WHEAT[1]

By J. G. Malloch[2] and R. Newton[3]

Abstract

A significant inverse relation was found between yield and protein content, more pronounced in 1930 than in 1931. Reduction of yield by removing tillers or heads increased the protein content in both these years and the weight per 1000 kernels in 1930. Grade and kernel texture were unaffected by this pruning.

Introduction

Yield and protein content are the most important characters of Canadian wheat, the former because of its direct effect on the farmer's revenue and the latter because of its relation to baking quality. Unfortunately, when wheat is grown under climatic conditions favoring high yield the protein content is usually low. Climate, however, is not the only factor affecting these characters, since it is well known that the yield will vary markedly in different parts of the same field and similar variations in protein content have been shown to occur (4). Waldron (5) has reported an inverse relation ($r = -0.556$) between the yield and protein content of 25 varieties of wheat grown in replicated plots in a single field. The experiments reported in this paper were designed to test the relation between yield and protein content of a single variety as affected by variations in the soil and by pruning the plants. The grade and weight per 1000 kernels were studied concurrently.

Effect of Soil Heterogeneity

The effect of soil heterogeneity on the yield and protein content of wheat was measured in 1930 and 1931. In both years the field selected was reasonably level and did not show any obvious variations in soil. The crop was a *pure line of Red Bobs 222 in 1930 and of Marquis in 1931 so that variation* in inherited characters was reduced to a minimum. Just prior to the harvesting of the main crop, fifty 18-ft. rows were cut by hand at locations scattered over the field. The field used in 1930 was approximately 14 by 34 rods; and that used in 1931 about 8 by 62 rods in size. Each 18-ft. row was threshed separately, the yield recorded and the grain preserved for protein determinations. The data are given in Table I and the statistical constants calculated from them in Table II.

It will be seen that yield was more variable than protein content and that the variation in yield was more pronounced in 1931 while the protein content varied more in 1930. The correlation and regression coefficients are significant.

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The significance of the difference between the values of *r* for the two years may be determined by means of the transformation due to Fisher (1, p. 182). The difference between these transformed values is 0.39 with a standard error of 0.206; this, though suggestive, cannot be regarded as unquestionably significant. The difference in the regression coefficients for the two years is 0.00475 with a standard error of 0.00131 giving a *t* value of 3.642, and therefore, is definitely significant.

TABLE I

YIELD (gm.) AND PROTEIN CONTENT (%, 13.5% MOISTURE BASIS) OF WHEAT FROM 18-FT. ROWS

Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein
				1930					
243	14.1	344	14.9	400	13.5	445	13.4	463	14.1
424	14.4	349	14.4	444	13.0	339	14.9	390	14.5
305	14.8	228	15.7	300	15.1	314	14.6	433	14.5
322	14.4	377	14.9	296	15.3	230	15.3	483	12.5
413	14.3	300	15.0	360	15.0	235	15.1	330	14.2
323	14.7	252	15.4	294	15.3	218	15.1	294	14.6
235	15.5	286	14.9	232	15.5	262	15.2	310	15.3
182	15.3	273	14.2	246	14.9	238	14.8	208	16.7
137	16.2	228	15.6	253	14.3	259	15.1	270	15.8
255	12.9	145	16.5	318	14.7	249	14.5	321	15.7
				1931					
223	13.3	501	13.7	393	14.3	304	14.0	514	14.5
373	14.3	175	13.9	319	14.2	306	13.5	229	14.7
303	14.1	349	12.9	314	14.4	178	14.4	151	14.7
342	14.8	303	14.2	185	14.5	294	14.1	193	15.4
298	14.7	297	15.1	94	15.0	236	14.1	181	14.6
373	13.6	232	14.7	164	15.4	212	14.5	178	14.2
426	14.5	45	14.8	239	14.6	273	14.8	234	14.8
419	14.0	143	14.7	90	15.0	226	14.8	262	14.6
376	13.6	357	14.4	353	14.4	418	14.4	230	15.2
326	12.9	363	14.1	342	14.0	390	14.1	316	15.3

Thus the correlation coefficients indicate that high yield was associated with low protein content, though less regularly in 1931 than in 1930. While this difference in closeness of association in the two years was just below the level of statistical significance, the data are not extensive enough to warrant a conclusion that the degree of association is

TABLE II

STATISTICAL CONSTANTS
(Data from Table I)

—	1930	1931
Average yield (gm.)	301	281
Standard error	80.3	102.2
Average protein (%, 13.5% moisture basis)	14.8	14.4
Standard error	0.83	0.56
Correlation (*r*)	−0.68	−0.42
1% point for 47 pairs	0.37	0.37
Regression (*b*)	−0.00709	−0.00233

statistically constant, and further experiments might easily produce significant differences. The regression coefficients show quite definitely that the amount by which a given change in yield affects protein content varies from year to year. In 1931, the decrease in protein content with increased yield was significantly less than in 1930.

Effect of Pruning to Reduce Yield

While both climate and soil, as well as other factors, such as varietal differences and irregularities in seeding, play a part in modifying the relation between yield and protein content in different seasons, the variations found in different parts of a level field in one season must depend mainly on soil differences. Of the principal nutrient substances, nitrates may be expected to exert the chief influence. Gericke (2) showed that yield and protein content are affected by the time at which nitrates become available. This depends largely on the relative rates of nitrification at different stages of plant growth. Nitrates may be used to build up the vegetative parts of the plant or for kernel formation. Since the weight of kernels produced is closely related to the amount of vegetative growth it follows that if the supply of nitrates in the soil is constant, any factor which depresses vegetative growth is likely to raise the protein content.

An experiment designed to test the above hypothesis was conducted concurrently with the experiment just reported. It was assumed that removal of parts of the top of the plant would not affect root development to any marked degree and that therefore the artificial reduction of yield by pruning should lead to a higher proportion, in the remaining grain, of materials absorbed from the soil. It is impossible to eliminate entirely variations in the supply of nitrates to each plant, but by use of small plots and the concentration of the experiment on a small area the chances of major variations are reduced. Further, by replication of the treatment in four blocks of plots and the arrangement of the plots in each block at random, only such soil heterogeneity as occurs within blocks will affect the treatment comparisons.

The treatments used, together with a short title for each, are given below:

Check—No treatment.

Tillers removed—All but two tillers were removed from each plant when normal tillering was completed and second growth tillers were cut back throughout the growing season.

Heads removed at flowering—All but two heads were removed at the time the upper heads were in flower. Any heads which appeared subsequently on late tillers were removed.

Heads removed in milk stage—All but two heads were removed when the kernels of the upper heads were in the milk stage.

The plots were arranged in four blocks, each treatment occurring once in each block. Each plot consisted of three rows 2 links apart, 29 links long in 1930 and 37 links long in 1931. As soon as all the plants had emerged the centre row of each plot was thinned until the plants were two inches apart.

The treatments were applied to the centre rows only. At harvest a 2-link border was removed from the ends of the plots and the centre rows were cut and threshed separately.

Protein Content

The protein content of the grain is shown in Table III and the statistical constants calculated from these results in Table IV.

The variance due to treatment and also to the interaction "Years × Treatments" significantly exceeds the error variance. The treatment variance, however, is not significantly greater than the foregoing interaction. In other words, pruning the plants definitely affected the protein content of the grain but the relative effect of the treatments was not the same in the two years of the experiment. The necessary difference between the means of four plots was calculated from Table IV and found to be 0.32. Turning to Table III, we find that in 1930 all of the treatments gave protein contents significantly higher than that of the check plots. Removal of tillers and removal of heads at flowering time increased the protein more than removal of the heads in the milk stage. The results in 1931 were different in character. Removal of the heads at flowering time did not increase the protein content significantly. The removal of tillers gave increased protein though not to the same extent as in 1930. The increase due to the removal of heads in the milk stage was identical in the two years. In 1931 there was not the significant difference between the treated plots that there was in 1930.

These results show that reduction in yield by pruning will increase the protein content, thus supporting the initial hypothesis. It should be noted that the pruned rows were in close proximity to rows of normal plants. It is possible that competition deprived the treated plants of part of their share of nutrients. The effects obtained can therefore be regarded as minimal.

TABLE III

EFFECT OF PRUNING ON PROTEIN CONTENT
(%, 13.5% MOISTURE BASIS)

	Check	Tillers removed	Heads removed at flowering	Heads removed at milk stage
		1930		
Block 1	15.6	16.3	16.6	15.6
Block 2	15.0	16.5	16.4	15.7
Block 3	15.1	16.3	16.0	15.7
Block 4	15.3	16.3	17.1	15.6
Average	15.2	16.3	16.5	15.6
		1931		
Block 1	14.3	14.7	14.7	14.9
Block 2	14.5	15.0	14.8	14.8
Block 3	14.8	15.2	14.8	15.3
Block 4	14.6	15.0	14.5	14.7
Average	14.5	15.0	14.7	14.9

(Necessary difference in yearly averages = 0.32.)

TABLE IV

ANALYSIS OF VARIANCE
(Data from Table III)

	Degrees of freedom	Sum of squares	Mean square	Z value	
				Calculated	At 5% point
Years	1	10.69	10.69	—	—
Blocks	6	0.56	0.093	—	—
Treatments	3	2.97	0.990	1.528	0.8138
Years × Treatments	3	1.79	0.597	1.275	0.8138
Error	18	0.84	0.0466	—	—
Total	31	16.85			

Kernel Texture and Grade

The variations in protein content were not reflected in the appearance of the kernels. In the two years of the experiment there was only one sample which had less than 99% vitreous kernels and this contained 96.5%. The official grades in 1930 varied from 1 Hard to 2 Northern, the majority of the samples being classed as 1 Northern. In 1931 the samples all graded 2 or 3 Northern. The differences in grading are attributable to differences in the maturity of the samples and therefore are irrelevant to the present discussion.

Weight per Thousand Kernels

Since the pruned plants were arbitrarily limited to two heads each, the vigor of vegetative growth could not be expressed in terms of total yield of grain. However, since the average number of kernels per plant would be nearly constant for all treatments, the weights per 1000 kernels may be taken as a good indication of vegetative vigor. These are given in Table V.

TABLE V

EFFECT OF PRUNING ON WEIGHT PER 1000 KERNELS
(gm.)

	Check	Tillers removed	Heads removed at flowering	Heads removed at milk stage
1930				
Block 1	36.8	42.8	36.7	38.2
Block 2	34.4	38.2	39.5	32.1
Block 3	36.9	42.7	37.4	36.8
Block 4	35.4	39.1	39.2	37.8
Average	35.9	40.7	38.2	36.2
1931				
Block 1	33.3	33.1	34.1	34.2
Block 2	29.7	30.2	30.0	32.3
Block 3	28.7	26.2	27.3	27.4
Block 4	33.3	32.0	29.7	31.4
Average	31.2	30.4	30.3	31.3

It does not require a statistical analysis to show that the differences in 1931 were not significant. The results for 1930 were however subjected to an analysis of variance, reported in Table VI. This shows that the treatments

had a significant effect in 1930, the necessary difference in weight per 1000 kernels being 1.46. Those plants from which tillers or heads had been removed at flowering gave heavier kernels than the plants in the check plots. The difference between the plants treated in the milk stage and the checks is not significant.

TABLE VI

ANALYSIS OF VARIANCE
(Weight per 1000 kernels, 1930)

	Degrees of freedom	Sum of squares	Mean square	*Z*
Blocks	3	16.64	5.55	0.1891
Treatment	3	58.98	19.66	0.8218
Error	9	34.20	3.80	
Total	15	109.82		

The treatments which gave significant increases in weight per 1000 kernels are those which gave the greatest increase in protein content. It is evident therefore that pruning affected the protein content by greatly increasing the nitrogen supply to the remaining kernels rather than by interference with the deposition of carbohydrates. The results show that conditions which favored nitrogen supply to the greatest degree also favored the laying down of carbohydrates, since the increase in weight of the kernels cannot be entirely accounted for by the increased weight of protein. The correlation between yield and protein in the first experiment and the effect of pruning on protein and on weight per 1000 kernels were all greater in 1930 than in 1931.

The results of these two experiments support the common observation referred to in the introduction that usually there is an inverse relation between yield and protein content. This does not mean however that yield can only be increased at the expense of a decrease in protein content. Under suitable conditions there can be a concurrent increase in both yield and protein (3). In any study of cultural practices for wheat their effect on protein content as well as on yield should be considered.

Acknowledgment

We are indebted to Mr. J. W. Hopkins of the Division of Biology and Agriculture, National Research Council, for his assistance with the statistical analysis of the data.

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IS *RORIDULA* A CARNIVOROUS PLANT?[1]

BY FRANCIS E. LLOYD[2]

Abstract

The genus *Roridula* has two species, the leaves of both of which are armed with numerous glands secreting a "balsam"-like exudate, whereby many insects are captured, simulating in this the behavior of *Drosera*, *Drosophyllum* and *Byblis*. But as Marloth held, these plants are not carnivorous.

Living "commensally" on both species are at least five different insects, three crab-spiders and two capsids (true bugs). All these can move about freely on the *Roridula* plants without danger of capture; they gain their livelihood by sucking the juices of freshly captured insects.

The structure of the glands of these plants and the nature of their secretion are described.

The immunity of the insects above mentioned from capture is attributed to the character of the surface of the resinous secretion, to the spiny surface of the insects, which prevents the presentation of extensive surfaces for adhesion and to the ability of the insects to negotiate their environment. The argument is not held to be conclusive but suggestive; and the matter is worthy of further study.

Under the above title a brief report was offered at the Leicester meeting of the Section of Botany, British Association for the Advancement of Science, and an answer given in the negative. It is the purpose of the present paper to give the evidence in support of that answer. That this is not superfluous is indicated by the fact that in general accounts *Roridula* is still cited as one of the relatively few carnivorous plants. Thus Quintanilha (8, Plate I) includes it in his roster. I had done so myself (4, Plate 16) but for the timely reception of material kindly afforded me by Professor von Wettstein of Munich, from the examination of which I found that the secretion of the glands is a resinous one,* and that their structure, hitherto incorrectly described by both Fenner (3) and Bruce (1, Plates 20, 21), is very different from that of the glands of *Drosera*. The veteran South African botanist, the late Dr. Marloth, who in later years came to the position that *Roridula* is not carnivorous, thought indeed that its inclusion in the Droseraceae is traceable to the general similarity of the plants to *Drosera*, and especially of the glands. As the genus is South African, with only two species, and is in general but little known, there has been little careful examination made of it, especially as it has only seldom been introduced into glasshouse cultivation. At the present moment, material of the two species may be found growing, so far as I know, only in the glasshouses of the Botanical Institute in Munich. It was this happy circumstance that enabled me to direct my own observation to specimens of both species (Plate I–1, 2) during the summer of 1933. Not only to specimens of the plants alone, however, but to one (Plate I–3) of the several species of insects that habitually live complacently on them without suffering the indignity of capture which overtakes other adventurous insects.

[1] *Manuscript received April 11, 1934.*
Contribution from the Department of Botany, McGill University, Montreal, Canada.

[2] *Macdonald Professor of Botany, McGill University.*

**The secretion persists as pellucid droplets indefinitely on dead and dried-up leaves, as Bruce* (1) *observed.*

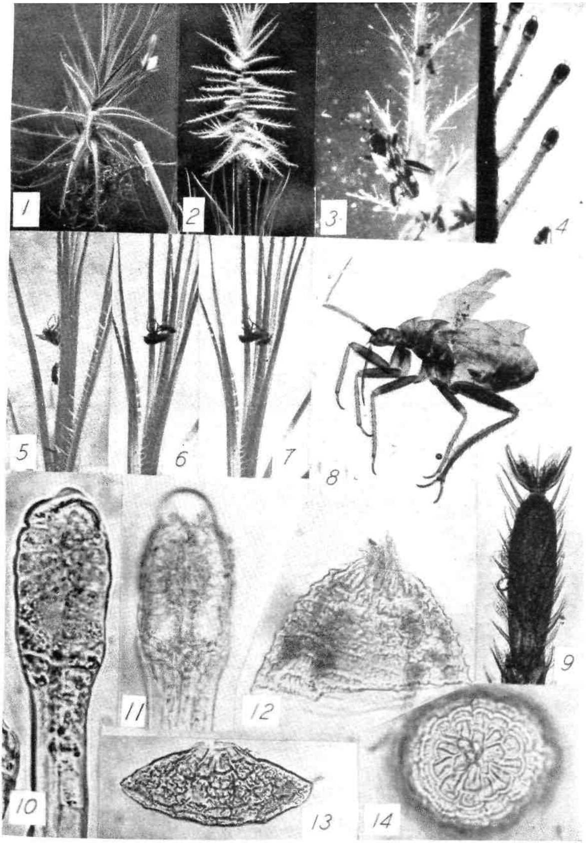

Fig. 1. *Roridula gorgonias. A capsid may be seen standing on one of the upper leaves.* FIG. 2. *R. dentata.* FIG. 3. *Portion of a leaf of R. dentata with a capsid (Pameridea sp.), for visualization of relative sizes. The insect was not alive.* FIG. 4. *Portion of leaf margin of R. dentata, with droplets of the secretion on the apices of the glands. The translucent spots near the bases of the glands indicate the secretion held in their canals.* FIGS. 5, 6 and 7. *Three views of the upper leaves of R. gorgonias showing a recently caught fly and a living capsid approaching, and in different positions sucking the juices of the prey.* FIG. 8. *The capsid in question, Pameridea sp.* FIG. 9. *A foot of this insect showing that all its parts are sharply pointed.* FIG. 10. *A small gland in optical section which shows the zone of large wedge-shaped cells near the base.* FIG. 11. *A small gland (but larger than that in Fig. 10), with the optical plane near the surface, showing a zigzag canal and the translucence produced by the resinous contents of the laterally viewed canals.* FIG. 12. *The cuticle removed from the apex of a large gland, showing the escape of cells destroyed by sulphuric acid through the apical pore.* FIG. 13. *The apex of a small gland showing the apical*

I am indebted to the late Professor K. von Goebel and to his successor, Professor von Wettstein, for the privilege of working on this material.

The course of thought on the question is briefly as follows. Marloth in 1903 (7) inferentially admitted *Roridula* to be carnivorous in saying, "We find that *Roridula* catches insects to obtain an additional food supply, but that a spider robs the plant of a share of the prey in spite of the sticky tentacles." In the following year Fenner (3), basing his view on the structure of the glands as he understood it, regarded them as "wirkliche, typische Insektenfaenger"* and in this Bruce (1, Plates 20, 21) concurred. "v. Marloth bestritten" is a pencilled remark made by Goebel on a margin of Fenner's paper, referring to Marloth's later view expressed in his beautiful Flora of South Africa (6). Here Marloth observed that the inclusion of *Roridula* with the Droseraceae would appear to be due to its apparent insectivorous habit. The plants, he now averred, are however not insectivorous, since *(a)* there are no digestive organs on the leaves; *(b)* the glands† are quite different in structure from those of *Drosera; (c)* the secretion is not a slime with digestive properties but a kind of balsam which yields, when washed with chloroform, a very viscid residue which contains about 10% of caoutchouc; and *(d)* the capturing of insects is accidental and of no advantage to the plant.‡ He showed further that there are four kinds of insects "commensal" on these plants, these including two species of bugs of the genus *Pameridea* (Capsidae) of which *P. marlothii* is found on *R. dentata* and the other, *P. roridulae*, on *R. gorgonias*,** and two spiders on *R. dentata*, *R. gorgonias* apparently being free of them. These spiders are "crab-spiders" (*Synaema* spp.), well known as lying in wait for insects inhabiting inflorescences of various kinds. The species of *Pameridea* are, Marloth believes, responsible agents for pollination, for they suck juices from the swollen portion of the stamen which contains sugar, whereupon the anther swings into the position of pollination.

In the light of the above conclusions we may now examine the situation as understood at present.

Structure of the Glands

There is one kind of gland only. In *Drosera*, in addition to the tentacles, there are minute sessile glands (4) which Darwin (2) thought might be absorptive. They have not been studied§ and nothing definite can be said about them. If Marloth was thinking of these we can concur with him. But it is now generally agreed that the chief digestive activity resides in the large glands supported on the ends of the tentacles, which are superficially yet

**It was Fenner's meaning that Roridula is a typical carnivorous plant; Goebel evidently so thought.*

†Marloth's figure was taken from Fenner.

‡Bruce's experiments leading to the opposite conclusion were few, equivocal and unconvincing.

***The single species seen in Munich seems to show no preference for one species.*

§Except anatomically by Fenner.

strikingly similar (Fig. 2) to those of *Roridula* (Fig. 1). In this genus, as Fenner states, they may be small and of simple construction, consisting of four rows of epidermal secreting cells surmounting the supporting tentacle, which contains no vascular tissue (Fig. 1–*B*); or, in the extreme, there may be a considerable number (± 16) of longitudinal rows of secreting epidermal cells surrounding a core of regularly composed parenchyma of a number of

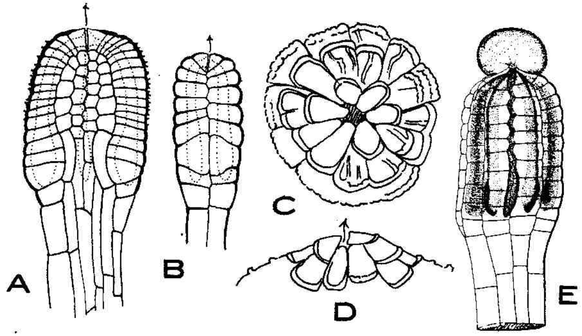

Fig. 1. *Roridula. A, Diagram of section of a large gland showing the core of parenchyma: the position of the resin canals is indicated by dotted lines, the middle canal seen as projected on the section (compare with E); B, section of a small gland with no core of parenchyma, resin canals indicated by dotted lines; C, apex of a gland seen from above; D, and as seen from the side; E, diagram of a medium sized gland of eight longitudinal series of epidermis cells. The extent of five of the resin canals is indicated by the stippled areas, also the secretion oozing from the apical pore.*

longitudinal series of cells according to the size of the gland (Fig. 1–*A*). The whole is covered by a thick cuticle (resistant to sulphuric acid), which displays much irregularity (ridging, folding) (Fig. 1), and in which he mistakenly believed there are pores suitable for the escape of the secretion, the nature of which escaped him. Another feature of structure was observed by him: that a transverse row of epidermal cells near the base of the gland is much deeper (in a longitudinal sense) than the neighboring, whereby the cells above and below are distorted, especially inwardly (Fig. 1 *A–B;* Plate *I—10, 11*).

In 1907, Bruce (1, Plates 20, 21) independently made a study of these glands and described the epidermal elements as being constricted in their middle, so that each cell was free of its neighbor except at top and bottom. Because each cell is constricted, both transverse and longitudinal sections of the glands show a circular intercellular space between adjacent cells. Now, as a matter of fact, this is true enough of the transverse section but not of the longitudinal, and Bruce was in error here.

The fact is, that between each two longitudinal series of epidermal cells there is a canal or duct which is much wider in the zone of the larger, near-basal cells, here forming, between each pair of large cells (which Bruce failed to see), a narrowly oval reservoir (in appearance), so that from the base of the gland there is a number of canals extending quite to the apex, in number equal to the number of series of epidermal cells (Fig. 1, *E*; Plate I—11). To complete this picture, there is at the apex of the gland a schizolytic opening through which the secretion, which fills the reservoirs and canals, escapes (Fig. 1; Plate I—12–14), demonstration of which I have already described (4, Plate 16). Because of the alternate emplacement of the hexagonal epidermal cells, the canals are always more or less zigzag (Fig. 1, *E*; Plate I—11). Escaping the observation in freshly mounted glands, the canals and reservoirs become readily visible after slight staining with Sudan III. It has been said that the epidermal cells are secretory and, by implication at all events, not the central parenchyma, but it is at the moment gratuitous to deny such activity to the central cells, in *Roridula* at least. It is perhaps less gratuitous to suggest that the major physiological activity in secretion is a function of the zone of wide cells against which lie the reservoir-like expansions of the resin canals (Fig. 1).

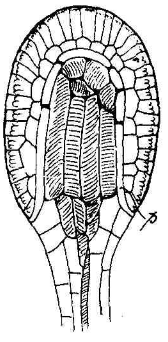

FIG. 2. *Section of the gland of Drosera for comparison with that of Roridula.*

The cases of *Drosera* and *Drosophyllum* are more convincing (3; 4, Plate 16).

Nature of the Secretion

Neither Bruce (who observed living plants at Edinburgh) nor Fenner suspected that the secretion was other than a watery slime. That this could not be the case I saw at once on examining leaves preserved in formalin, sent to me by Professor von Wettstein, for in this the drops of secretion were still intact, which had not been the case were it similar to that of *Drosera* (4, Plate 16). Simple tests showed the secretion to be of resinous nature. Marloth (6) had already examined it in this way, as above noted, finding a small proportion of caoutchouc.* Obtaining sufficient material at Munich, I extracted about a dozen leaves with acetone, which yielded a resin (or mixture of resins). The leaves were then extracted with petroleum ether by Dr. R. D. Gibbs, yielding an acetone insoluble material having properties of caoutchouc, so far as visible behavior indicated. We may thus agree with Marloth at least far enough to say that the secretion contains a caoutchouc or caoutchouc-like substance. The secretion as a whole is, at all events, very sticky

**Marloth mentions the use of Roridula as a fly-paper in the manner that Drosophyllum is used in its place of habitation.*

and, bird-lime fashion, captures very many insects, but there is no contributory action of movement in the tentacles (Bruce; Professor Adamson, in a personal letter).

"Commensal" Insects Not Captured

In view of the crowded emplacement of the glands (Bruce, Fenner) and of the great stickiness of the secretion which is abundant on the glands, it is a matter of no small surprise that any insects at all can negotiate the terrain with immunity. How do they do it, is the blunt question which shall now be answered, but perhaps only in part. There are at least five insects which can do so; four above mentioned, and an additional spider observed by Professor R. E. Adamson, according to personal notes received from him recently.

The following observations were made in Munich on the behavior of one of the capsid insects mentioned by Marloth. It came from South Africa along with living plants consigned to the Botanical Institute; evidently Goebel's doing, for his well known interest in carnivorous plants prompted him to gather everything possible from all parts of the world. That some of the insects shared the adventure was a matter of chance, but there they are and they breed and thus replenish their kind from year to year. In August, 1933, there were some half-dozen mature, and twice as many more young, insects. I was thus afforded an opportunity to get some light on the matter of their immunity to the apparent dangers which surround them. This was done chiefly by direct observation, but partly by experiment of sorts.

As regards the contribution of the capsids to the act of pollination, I was able to add little to, or to detract from, Marloth's account. Only a few flowers were available, one at a time. It was observed that only young insects visited the open flowers, and it may very well be that they find sufficiently delicate tissues present to permit them to, and that they do, suck their juices. It is certain, however, that they do not depend upon them for food. I found both old and young insects getting sustenance elsewhere after the flowers had passed. This part of the problem awaits more sustained study where material is sufficiently plentiful.

Aside from this it seems certain that the capsids do not derive any food directly from the plant, and it would indeed surprise us if the spiders (of which three species have been observed by Professor Adamson) are not in the same case. I kept a mature insect (a capsid) in a vial supplied with fresh leaves for several days, during which it probably became anhungered, but I was never able to observe it sucking juices from the leaves. It finally died with food in sight which it had not been able to appropriate. It seems clear that Marloth is quite correct in the view that the relation is commensal—or perhaps we should say mensal, the plant providing a well spread table and the insects being unbidden guests.

But it is a table with menace as well as provision. An unfortunate insect is caught (Plate I—5–7). On a leaf some distance off a capsid spies its flutter-

ings and is not slow to be present for the feast. But in order to come by it, it must pass over many rows of barrier tentacles (Plate I—1–4), which it does swiftly and with no let or hindrance. Not incredulous, for there was the evidence of my own eyes, but most curious, I experimented repeatedly, in a very simple fashion to be sure, by presenting a probe (a needle or fine sedge blade, etc.) to a resting and quiet insect; never was it caught napping, and while yet the menace was far off it would run to hide itself in another position. This repeatedly: up and down both faces of a leaf, around its edges, up the stem and down it (since the leaves are sessile the worst place of all), or out to the farthest tip of a leaf where nothing but tentacles are, and never once hesitating or stumbling from impediment. It is uncanny. Yet there must be an explanation, as there is of the ability of water-skippers to negotiate the water surface. But what is it?

We should note first that it is not because the insect cannot become entangled. I have several times seen my imprisoned specimen, moving slowly in the unusual surrounding of a glass vial, place its foot on a gland and, finding that its claws were sinking in the secretion, withdraw it, pulling out a sticky thread. This it did patiently, lifting the leg higher till the sticky thread broke. The insect then cleaned its foot by rubbing it with other feet till it was clean. Had its foot been supplied with a broad pad (Plate I—9), it is doubtful whether it would have had the strength to pull it away. Since the whole of the capsid's body (and wings) is armed with a covering of fine bristles (Plate I—8), it, like its foot, always presents a minimum surface to the sticky secretion. To test the efficacy of this covering, I endeavored again and again to catch the insect by quickly hitting its back with the glandular edge of a leaf, and was never able to do so. It would be dangerous to say that the immunity of the insect depends solely on this. Possibly many another kind of insect actually captured has a similar armament in which it has no help. There may therefore be something more to the explanation.

That it has long legs from the knees down, raising the body above the tentacles, is probably a factor. The insect can keep its underbody free of the tentacles during movement, though it is not very evident from observation that it never touches its back on them. It occurs to one that there may be some secretion from the insect which repels the glandular resins of the plant but, whether true or not, this is certainly not true of a dead insect.

But again, whatever its equipment for managing the situation, it does require management. The directness and swiftness of the insect's movements have persuaded me that it knows its job. If it does get caught, at least by its foot, it does not blunderingly fall into a panic but carefully behaves with circumspection—or at least it has seemed to me to do so—with the happy result of freedom from capture. Normally, its movements are not slow except while changing its stance while feeding. At other times it usually keeps to the upper or lower surface of the leaf where it is free from glands. When actually feeding, it could often place a foot on a gland unless watchful not

to do so. It may be supposed that the slower its movements the more circumspection could be employed, when it would probably act as my imprisoned specimen did.

Aside from this, the movements are swift and I conceive that this in itself contributes to immunity, for if it treads the glands rapidly its feet do not stick, I believe. Experiment indicates this. Placing a gland under the microscope, it was found that the surface of the gland can be touched with a needle point without its adhering. It was only when some pressure forced the point into the secretion that it adhered. Pulling out a thread of the gum, this usually broke as it did when I observed the insect freeing its caught foot in the vial.

The furniture of the foot with spines and pointed pads (Plate I—9) gives color to the view that the foot can be planted for a brief moment on a gland without danger.

This indicates that the *surface* of the secretion is scarcely at all adhesive. As may be expected, if it is composed of a mixture of resins and caoutchouc (or something akin), and if it contains some water, as it probably does, there will be a surface concentration of less adhesive material which must be broken through before the full effectiveness of the gum is displayed. Thus the insect may be pictured as treading on thin ice, the success of which is in not staying too long in one spot. That this explains also the immunity of the spiders seems equally true, since they, the crab-spiders, are habitually swift of movement. That other insects get readily caught we may suppose is owing less to their size than to the extent of surface for the adherence of the gummy secretion, and their alarm and panic on being caught but a little, leads to consequent floundering to make matters worse.

The spiders, Professor Adamson tells me, live in numbers on every leafy head of the plant. They build among the leaf bases their webby nests, to which they retire in waiting for prey to be caught on the leaves, when they dash out to devour them. The capsids usually rest on the upper or under surface of a leaf, but not exclusively, and soon spotting a caught insect they proceed to suck its juices. When this occurs, the young which are scattered here and there rally around and partake for themselves. Their movements are now quite leisurely. The illustrations show characteristic positions (Plate I—5–7).

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A SINGLE FACTOR MUTATION IN *MELILOTUS ALBA* DESC. HAVING MULTIPLE EFFECTS ON HOMOLOGOUS STRUCTURES[1]

By L. E. Kirk[2] and J. M. Armstrong[3]

Abstract

The multiple effect of a single factor mutation in *Melilotus alba* which affects several diverse characters of the plant including shape of leaflets and petals, position of the staminal column, morphology of the pistil, female fertility and vigor of growth, is described.

The results are significant in showing that one of the genes which presumably determined leaf shape in the species ancestor still continues to modify the shape of those organs which have evolved from the leaf.

It is now generally recognized that factor expression is dependent on the whole genetic complex of an individual and that all factors contribute to a certain end result. On the other hand it is equally clear that in many cases a single factor has multiple effects. In some cases this multiple or pleiotropic effect is apparent in a character, such as pigment development, affecting the whole organism. In other cases the multiple effect is seen on characters which are apparently unrelated. In the present case the effect is apparent on characters which are commonly believed to be phylogenetically related or homologous. Accompanying either of these phenomena due to pleiotropic factors, there may be a disturbance of the physiological or reproductive functions of the organism resulting in decreased vigor of growth and sterility.

An example of several characters being affected by a general pigmentary factor was noted by Mendel in his studies with sweet peas. He observed that plants with purple flowers had red spots in the axils of the leaves and bore brown or gray seeds, while in white flowered plants the red axillary spots were absent and the seeds were white. Tammes (4) records a similar effect of a general pigmentary factor in *Linum usitatissimum* where some of the factors affecting petal color were found to affect anther and seed coat color as well.

Hallquist (1) noted in *Lupinus angustifolius* the pleiotropic effect of a single factor upon unrelated traits where a factor for red flower color increased plant height and induced anthocyanin formation in the stems, and such plants bore seeds with glabrous shiny coats. Morgan *et al* (3) have recorded several cases in *Drosophila* of the manifold effect of a single factor which was frequently accompanied by lowered viability and sterility. One factor which determined rudimentary wings caused a shortening and twisting of the legs as well as female sterility. If crowding occurred or if food conditions were poor the larvae of rudimentary flies died off and in consequence the rudimentary class was smaller than expected.

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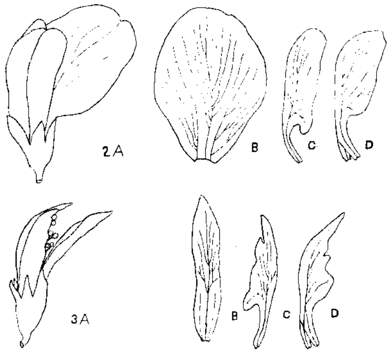
A mutation in common white blossomed sweet clover has recently been noted which affords an excellent example of the multiple effect of a single factor on the morphology of the organism accompanied by lowered vigor and sterility. This mutation has been named "Cutleaf" from its most noticeable character effect.

Morphology of the Cutleaf Mutant

The most obvious character affected by the mutant factor is the shape of the leaflets. In normal plants the leaflets are obovate in shape with finely dentate margins. In the mutants the shape of the leaflets is modified to linear while the margins are more coarsely dentate. The petiole of the central leaflet is somewhat bent. These differences are illustrated in Fig. 1. In the course of development the mutant leaves always remain more or less rolled up at the margins, while in the normal plants the leaves are flat and open.

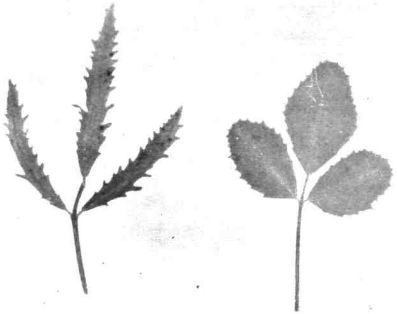


FIGS. 2 and 3. *Camera lucida drawings showing flowers and corolla parts of the normal plant and Cutleaf mutant. 2A, normal flower; 2B, 2C and 2D, standard wing and keel respectively of a normal flower. 3A, Cutleaf flower; 3B, 3C, and 3D, standard, wing and keel respectively of a Cutleaf flower.*

The second character affected is the shape of the corolla parts. Here, as in the case of the leaflets, the petals are much narrower than in normal plants (Figs. 2 and 3). Analogous to the change in leaflet dentation, the keel and wings differ markedly from the normal in having deeply lobed margins.

As a direct consequence of this reduction in the keel and wings the staminal column in mutant flowers emerges from the keel soon after the opening of the flowers (Fig. 3-A). In normal flowers, on the other hand, the release of the tension on the staminal column is brought about by a slight force, usually applied by the proboscis of an insect, and until this force is applied the column remains within the keel.

A fourth difference concerns the pistil. This difference can only be observed microscopically. Fig. 4 shows cross sections through the style and ovary of a normal and a Cutleaf pistil. It is seen that the normal pistil is completely closed, while in the mutant one side has failed to fuse, leaving a longitudinal slit. This slit is located opposite the main vascular bundle or at what may be regarded as the margin of the sporophyll.



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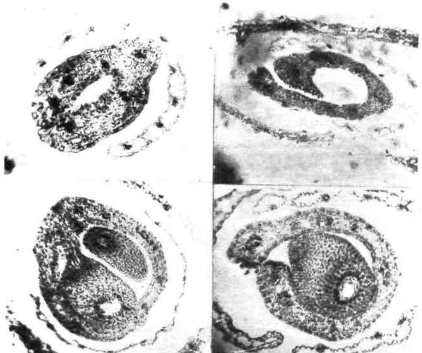

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FIG. 1. *Photograph of leaves of Cutleaf and normal plants. Cutleaf to the left and normal to the right.*

FIG. 4. *Photomicrographs of cross sections of the styles and ovaries of normal and Cutleaf pistils. Normal style and ovary to the left and Cutleaf style and ovary to the right.*

The mutant factor thus operates to modify the shape of the leaflets, the petals and the pistil. It also produces accessory effects on the vigor of growth and fertility. The mutant plants when grown in pots of the same size as those used for the normals attain to only about one-half the size. This may be attributed to a decreased rate of metabolism due to the reduced leaf area. The mutant plants flower abundantly but are completely sterile when either their own pollen or that of normal plants is used.

A list of the various characters affected is given in Table I, together with a comparison of normal and mutant plants.

TABLE I

A COMPARISON OF THE CHARACTERS IN NORMAL PLANTS AND IN CUTLEAF MUTANTS

Character	Normal	Cutleaf	Character	Normal	Cutleaf
Leaflet shape	Obovate	Linear	Position of staminal column in newly opened flowers	Within the keel	Outside of the keel
Leaflet margin	Finely dentate	Coarsely dentate			
Petiole of central leaflet	Straight	Bent	Pistil	Entire	Slit longitudinally
Posture of leaflets	Open	Rolled at margins	Vigor of growth	Normal	Greatly reduced
Petal shape	Obovate	Narrow	Fertility	Normal if flowers are manipulated	Completely sterile

Inheritance

Genetic data were obtained by growing progenies of heterozygous plants to the late seedling stage when the two types of segregates could be readily classified. A few plants from each line were then transferred outside in pots in the late autumn of 1933 and exposed to frost for a fortnight to break the biennial resting period. They were then brought back into the greenhouse and cytological and histological studies were made when they reached the flowering stage.

The factor determining morphological changes associated with the Cutleaf character was found to be completely recessive to its normal allelomorph, heterozygous plants being indistinguishable from homozygous normals. As seen from the data presented in Table II, the inheritance is obviously monofactorial. From six out of the seven heterozygous plants tested, normal and Cutleaf progeny appeared in a fairly satisfactory 3 : 1 ratio. Considering each progeny separately the departure from a 3 : 1 expectation is not significant but in all there is a slight but consistent deficiency of the recessive class. This may be due to a lower viability of the seeds in this class since a small percentage of the seeds sown failed to germinate; or it may be due to certation, a decreased rate of pollen tube growth in those microspores of the heterozygous plants which carry the Cutleaf factor.

The ratio obtained in the progeny of Plant 4 differs markedly from that in any of the other families. Among possible explanations may be considered the occurrence of additional new mutations of the Cutleaf factor and trisomic inheritance. The first presupposes a high mutation rate, which is highly improbable; the second was tested by cytological examination of ten normal and two mutant progeny plants, and all showed the normal chromosome number in somatic cells ($2n = 16$). The possibility of Plant 4 being an aneuploid ($2n + 1$) may therefore safely be ruled out. Finally, of course, there is always the chance that a few seeds from a heterozygous plant had become accidently mixed with seeds from a normal plant. But whatever may be the correct explanation, the occurrence of the two recessive mutants in this family of 102 plants does not prejudice the monofactorial hypothesis.

TABLE II

SUMMARY OF F_2 RESULTS ON THE INHERITANCE OF THE CUTLEAF CHARACTER IN SWEET CLOVER

Plant	Normal	Cutleaf	No. of seeds sown
8	150	46	200
9	150	48	200
10	153	45	200
5	33	8	43
7	40	7	50
11	44	13	60
4	102	2	106

Sterility

Kirk and Stevenson (2) showed that *M. alba* is quite variable with respect to self-fertilization. Some individual plants set pods freely both in the field and in the greenhouse, while others, although setting a small proportion of pods in the field produce pods in the greenhouse only after manipulation of the flowers. The normal segregates of the writers' material belong to the second type and fail to produce a single pod in the greenhouse until the flowers are manipulated, in which case pod-setting is fairly good. The Cutleaf mutants proved to be completely self-sterile with or without flower manipulation. An investigation was made to determine whether this was attributable to the male or female organs.

Cytological examination of mature pollen of the normal and Cutleaf plants showed that in both cases it was morphologically very good, the normal giving $94.8 \pm .28\%$, and the Cutleaf $95.4 \pm .55\%$. Germination of the pollen was tested in a culture medium consisting of 12% of sucrose, 1% of agar and 89% of distilled water at a temperature of 27° C. In four hours the pollen of all flowers tested showed germination ranging from 60 to 70%. In another test the Cutleaf flowers were manipulated to insure that the pollen would reach the stigma and another set of flowers was pollinated with normal pollen. In both cases the pollinations were completely unsuccessful. These facts appear to indicate that the sterility is not due to the lack of good viable pollen but rather it must be attributed to the defective pistils which have been previously described.

Discussion

The Cutleaf factor mutation responsible for the alteration of leaflet morphology and for the associated changes in the petals and pistil affords an example analogous to the pleiotropic factors, reported in genetic literature, which control general pigment development. If we presuppose a dominant factor for normal shape of leaves, any mutation of this factor might also affect those plant organs which have evolved from leaves.

In the *Pteridophyta* there are many species in which the leaves not only carry on the normal vegetative functions but are spore-bearing organs as well. The order *Filicales* contains a graded series of genera showing the various steps by which certain leaves are set aside as spore-bearing organs or sporophylls, while others retain only the vegetative function. This differentiation of the frond into vegetative leaves and sporophylls reaches a high point of development in horsetails and club-mosses and is the rule in seed plants.

There are doubtless many factors which determine independently the shape of leaves, petals, stamens and pistil, but it is remarkable that a single factor should markedly influence the morphology of all of them at the same time. The mutation described here, with its manifold effect on the leaves and various derived organs, indicates that in *M. alba* there is at least one factor which in the course of evolution has retained a general control over the shape of those organs which have evolved from the primitive leaf.

Although the unbroken association of modified characters in the mutant which are inherited in a simple monofactorial manner indicates a single gene modification, yet the possibility of aneuploidy or chromosome translocation must not be overlooked. The addition to or subtraction from the genome of a single chromosome has been shown in many plants to be effective in disturbing the genic balance resulting in a departure from the normal expression in many characters. The normal chromosome number ($2n=16$) and regular chromosome behavior at meiosis were invariably found in the several plants of the two segregating classes which were examined cytologically. Hence the cytological evidence substantiates the hypothesis of a single gene change.

The other changes, *i.e.*, lowered vigor of growth and sterility, which characterized the mutant plants may be regarded as accessory effects of the morphological modifications. The genetic data presented in Table II show a slight but consistent deficiency of the recessive class. This may be ascribed to certation or to a slightly lowered viability of the seeds. Considering the reduced vigor of the Cutleaf plants, the latter explanation seems the more probable. The pollen produced on the recessive mutants was shown to be as good, and capable of as high percentage germination, as that produced on normal plants, hence it is probable that they are female sterile owing to defective pistils.

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THE EFFECT OF TEMPERATURE UPON THE PRE-ADULT LARVAE OF THE BULB NEMATODE *ANGUILLULINA DIPSACI* (KÜHN, 1858) GERV. AND v. BEN., 1859, IN RELATION TO TIME AND MOISTURE[1]

By R. J. Hastings[2] and W. Newton[3]

Abstract

In a moist environment, a minimum exposure of 120 min. at 110–113°F. is required to destroy pre-adult larvae of the bulb nematode *Anguillulina dipsaci* (Kühn, 1858) Gerv. and v. Ben., 1859, but progressively shorter exposures are required as the temperature is raised. At 116.5–118.5°F. the lethal exposure is 60 min. and at 118.5–120°F. an exposure of 30 min. is required.

In a dry environment exposures of 150 min. to temperatures as high as 140°F. are not lethal to pre-adults and the heat treatment does not affect their ability to induce the characteristic symptoms of infestation in barley seedlings.

The pre-adults are more resistant to heat than any other stage in the life history of the nematode.

The data suggest that the ineffectiveness of the standard hot water treatment when applied late in the season is due to the fact that the major development of pre-adults takes place after the bulbs are lifted, and also because the masses of dormant pre-adults are often well isolated from the moisture of the bath by the bulb scales and corky basal plates, and are more resistant to heat in a dry, compared with a moist, state. It is recommended that the hot water treatment be employed not later than four weeks after lifting when the lifting is done as soon as the foliage dies down.

A pre-soak is suggested as a possible means of increasing the effectiveness of the standard hot water treatment.

The hot water treatment as developed by Ramsbottom (4) and others (5, 6) for the destruction of the parasitic nematode *Anguillulina dipsaci* in narcissus bulbs has been of inestimable value to the bulb growers, yet the writers' experiments and those of others (2, p. 37) show that the treatment frequently fails to destroy all the nematodes, particularly when the bulbs are treated after a long storage period. To account for the discrepancies between the results of different investigators as to the time required to kill the bulb nematode at 110°F. and for the fact that the nematodes are more resistant in bulbs that have dried out, the writers suggested that certain stages in the life history were more resistant than others, and data were presented which proved that pre-adult larvae were less resistant when moist than when dry (1). No previous investigator has taken into consideration the fact that the nematodes in bulbs are not surrounded by water, hence the lethal time and temperature of treatment for nematodes within bulbs are not likely to be the same as those determined when the nematodes are suspended in water.

Experimental

The nematodes used in these experiments were entirely the pre-adult larvae obtained as "wool" from diseased narcissus bulbs. The wool consists

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of conspicuous masses of tightly coiled nematodes, ranging in size from 860 to 1150 μ, which when placed in water may take three to four hours or longer to revive and become motile. The length of time required by the nematodes to revive appeared to vary directly with the time that they had been in the dormant desiccated condition.

The effect of temperature in relation to time and moisture was determined as follows:—Pieces of the wool consisting of about 1000 nematodes were placed in small vials, 2.5 cm. in length, made from thin glass tubing 4 mm. in diameter. The moist environment was created by introducing a drop of water into the vials. The vials were then plugged with rubber stoppers and dropped into a beaker of water maintained at the desired temperature. Upon removal from the hot water, the contents of the vials were shaken out into syracuse dishes of cold water and kept for 24 hr. before microscopic observations were made. Finally, the water suspensions of heated nematodes were used as inoculum by adding them to sterilized soil in 3-in. pots which were seeded to Barks barley. The presence or absence of the raised white spots on the leaves, the characteristic infestation symptoms in the barley seedlings, served to evaluate the influence of temperature and exposure upon the inoculum. The results are shown in Tables I to IV.

TABLE I

THE PERCENTAGE OF MOTILE NEMATODES AFTER EXPOSING MOIST PRE-ADULT LARVAE TO TEMPERATURES OF 110–120° F. FOR DIFFERENT PERIODS OF TIME

Temp., °F.	Exposures, min.								
	30	45	60	75	90	105	120	135	150
110–113	50	10	10	1	0.5	0.5	0	0	0
113–115	10	1	1	0.5	0.5	0	0	0	0
115–116.5	5	5	5	0.5	0	0	0	0	0
116.5–118.5	0.5	0.5	0	0	0	0	0	0	0
118.5–120	0	0	0	0	0	0	0	0	0

TABLE II

THE AMOUNT OF INFESTATION (%) IN BARLEY SEEDLINGS INDUCED BY PRE-ADULT LARVAE EXPOSED TO HEAT FOR 30 AND 60 MIN. IN A MOIST ENVIRONMENT

Temp., °F.	1st sowing, Oct. 7		2nd sowing, Oct. 28		3rd sowing, Dec. 14	
	Exposure		Exposure		Exposure	
	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
110–113	0	0	30	10	75	20
113–115	0	0	10	5	60	50
115–116.5	0	0	10	10	45	15
116.5–118.5	0	0	10	0	35	10
118.5–120	0	0	0	0	5	5
Check—Untreated nematodes	0		30		50	

TABLE III

THE PERCENTAGE OF MOTILE NEMATODES AFTER EXPOSING DRY PRE-ADULT LARVAE TO TEMPERATURES OF 110–140° F. FOR DIFFERENT PERIODS OF TIME

Temp., °F.	Exposures, min.						
	30	45	60	90	105	120	150
110–113	100	100	100	50+	50+	50+	50+
113–115	100	100	100	50+	50+	50+	50+
115–116.5	100	100	100	50+	50+	50+	50+
116.5–118.5	100	100	100	50+	50+	50+	50+
118.5–120	100	100	100	50+	50+	50+	50+
129–131	90	90	75	50+	50+	50+	50+
138–140	75	75	75	50+	50+	50+	50+

TABLE IV

THE AMOUNT OF INFESTATION (%) IN BARLEY SEEDLINGS INDUCED BY PRE-ADULT LARVAE EXPOSED TO HEAT FOR 30 AND 60 MIN. IN A DRY ENVIRONMENT

Temp., °F.	1st sowing, Oct. 7		2nd sowing, Oct. 28		3rd sowing, Dec. 14	
	Exposures		Exposures		Exposures	
	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
113–115	10	15	10	10	75	75
115–116.5	10	0	10	50	50	55
116.5–118.5	0	0	50	60	50	40
118.5–120	10	15	30	60	60	60
129–131	0	0	20	70	65	60
138–140	0	0	10	10	55	20
Check—Untreated nematodes	0		30		50	

The data presented in Table I show that in a moist environment, an exposure of 120 min. is lethal at 110–113°F., and the time required to destroy the pre-adult larvae progressively becomes shorter as the temperature is raised. At 116.5 to 118.5°F. a 60 min. exposure is enough, and at 118.5–120°F., only 30 min. is required.

The data in Table II suggest that the exposures given in Table I are minimum values, for there was a survival of nematodes after an exposure of 60 min. at 118.5 to 120°F., and these survivors were capable of infesting barley seedlings (Fig. 1).

The data in Table II also show that successive sowings of barley are sometimes required before the characteristic symptoms of nematode infestation develop in the barley seedlings. As pointed out in a previous publication (3) barley seedlings develop characteristic symptoms only when the environment favors a stocky growth.

The data in Tables III and IV show that in a dry environment temperatures as high as 140°F. for 60 min. are not lethal to the pre-adult larvae, and that the heat treatment does not prevent them from inducing the characteristic infestation symptoms in barley seedlings (Fig. 2).

The nematode "wool" consists entirely of pre-adults and it will be seen from the data in Table V that this stage is much more resistant to heat than younger stages and slightly more resistant than adults. A mixture of adults, pre-adults, half-grown and very young larvae together with eggs, was teased out of green narcissus leaves and water suspensions were subjected to a temperature of 110–112°F. for 30 and 60 min. These were examined six hours later for motility, and again 96 hr. later for the possible presence of freshly hatched larvae from viable eggs. The percentage motility in each class was based upon a count of not less than 50.

TABLE V

THE EFFECT OF EXPOSING VARIOUS STAGES OF NEMATODES TO A TEMPERATURE OF 110–112° F.

Exposure, min.	Stages and average size				
	Adults, 1300–1400 μ	Pre-adults, 720–1200 μ	Half-grown larvae, 570–720 μ	Very young larvae, 340–570 μ	Eggs
	% Motile				% Viable
30	18	19	0	0	0
60	4	10	0	0	0

Conclusions

These results have an important bearing upon bulb sterilization by immersion in water at 110–112°F. Owing to the greater resistance to heat of pre-adults compared with other stages in the life history of the organism, it is apparent that the treatment should be given as early as possible, for the major development of pre-adults takes place after the bulbs begin to ripen. Furthermore, bulbs frequently lose considerable water in storage, and the writers' results show that dry pre-adults are more resistant than those that have been activated by moisture. The "wool", or masses of pre-adult larvae, begins to form under dry storage conditions shortly after the bulbs are lifted, and masses of dormant pre-adults are often well isolated from the moisture of the bath by the corky basal plate and the bulb scales, although the conspicuous "wool" is usually observed around the exterior of the basal plate. Under the climatic conditions of the coast of British Columbia, these masses of dormant pre-adults have been found after six weeks of storage, hence it is recommended that the bulbs should be passed through the hot water not later than four weeks after lifting, when the lifting is done as soon as the foliage dies down.

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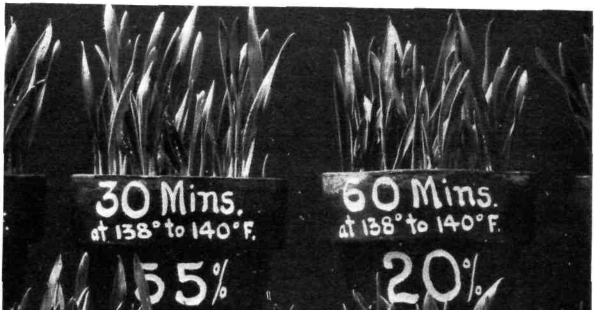

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FIG. 1. *The amount of infestation in barley seedlings induced by pre-adult larvae exposed to 118.5–120° F. for 30 and 60 min. in a moist environment.*

FIG. 2. *The amount of infestation in barley seedlings induced by pre-adult larvae exposed to 138–140° F. for 30 and 60 min. in a dry environment.*

The writers' conclusion that the hot water treatment should be employed early rather than late is supported by field studies. A Vancouver Island grower treated one lot of King Alfred bulbs in early August and the remainder in October. No infestation was found in those treated in early August, while 30% were found infested in those treated in October.

In view of the marked resistance of the dormant pre-adult larvae of *Anguillulina dipsaci*, particularly when in a dry condition, it is probable that the hot water treatment of other bulbs, plants and seeds in which the nematodes occur internally, may have to be re-investigated. The lowering of the resistance to heat of pre-adult larvae through absorption of water suggests the advantage of a pre-soak before the immersion of bulbs, plants or seeds in hot water.

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A COMPARISON OF THE ROUTINE RAPID WHOLE BLOOD (STAINED ANTIGEN) AND THE ROUTINE RAPID SERUM AGGLUTINATION TESTS FOR PULLORUM DISEASE[1]

By Jacob Biely[2] and W. Roach[3]

Abstract

Data are presented on 4,429 birds, comprising eight flocks, which were tested for pullorum disease by the whole blood agglutination test and the rapid serum agglutination test (commercial laboratory). The diagnoses agreed in the cases of 4,046 birds (97.24%) and disagreed in the cases of 122 birds (2.75%).

Of the 122 birds, 43 were diagnosed as positive by the whole blood agglutination test and as negative by the rapid serum agglutination test, while 79 were diagnosed as positive by the rapid serum agglutination test and as negative by the whole blood agglutination test.

Of the 122 birds, 102 were retested by the whole blood, rapid serum (Laboratory 1), and rapid serum agglutination test (Laboratory 2, (Experiment Station Laboratory)).

There was a closer agreement between the diagnoses made on the basis of the whole blood and rapid serum tests (Laboratory 2) than between those made on the basis of the rapid serum (Laboratory 1) and rapid serum (Laboratory 2) tests (71.56% and 62.37% respectively).

A detailed study of the retests and post-mortem examination of the 102 birds is presented.

In previous papers (2, 3) the writers reported on the comparative accuracy of the whole blood agglutination test (stained antigen), rapid serum and tube agglutination tests for the detection of carriers of pullorum disease. Papers published by other investigators confirm the conclusion of the writers that the whole blood agglutination test, when applied by a trained technician, provides an accurate, rapid and efficient means of diagnosing pullorum disease.

The purpose of the present paper is to report the results obtained from a comparative study of the routine whole blood agglutination test as conducted in the field, with the routine rapid serum agglutination test as conducted by a commercial laboratory.

Material and Methods

The whole blood agglutination test was carried out by four persons—*A*, *B*, *C* and *D*,—the last, *D*, instructing the others in the technique and interpretation of the tests, as the testing proceeded. The methods followed were those already described by Biely and Roach (2). Simultaneously with the drawing of a drop of blood for the whole blood test, blood samples were taken for the rapid serum test. These samples were shipped at once to a commercial laboratory nearby, where they were tested by the usual technique employed in the rapid serum agglutination test.

Data

Data were collected on 4,429 birds, comprising eight S.C.W. Leghorn breeding flocks. In every one of the eight flocks, positive reactors were found,

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[2] *Investigation conducted privately by the senior author while employed on a special problem in poultry diseases at the University of British Columbia.*

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thus providing excellent material for a comparative study of the whole blood and the rapid serum agglutination tests. The results are shown in detail in Table II, and are summarized in Table I.

TABLE I

SUMMARY OF RESULTS

	No.	%
Positive to the whole blood and positive to the rapid serum	261	5.89
Negative to the whole bood and negative to the rapid serum	4046	91.35
Negative to the whole blood and positive to the rapid serum	79	1.78
Positive to the whole blood and negative to the rapid serum	43	.97

Association coefficient according to the method of Yule (4) = 0.994.

TABLE II

COMPARISON OF RAPID WHOLE BLOOD (STAINED ANTIGEN) AND RAPID SERUM AGGLUTINATION TESTS FOR PULLORUM DISEASE

Technician by whole blood test	Group	No. tests	Number of diagnoses in which both tests agreed				Total agreement		Number of diagnoses in which the tests disagreed				Total disagreement	
			Negative		Positive				P—Whole blood N—Rapid serum		P—Rapid serum N—Whole blood			
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	1	264	255	96.59	6	2.26	261	98.8	2	0.75	1	0.38	3	1.13
	2	320	316	98.75	3	0.93	319	99.6	1	0.31			1	0.31
	3	251	247	98.41	1	0.39	248	98.8	3	1.19			3	1.19
	4	290	284	97.93			284	97.9	1	0.34	5	1.72	6	2.06
	5	284	213	74.99	64	22.57	277	97.5	3	1.05	4	1.41	7	2.46
B	6	167	162	96.96	4	2.25	166	99.4	1	0.59			1	0.59
	7	411	407	99.02	1	0.24	408	99.46	1	0.24	2	0.48	3	0.73
	8	456	414	90.78	22	4.82	436	95.61	3	0.65	17	3.72	20	4.38
	9	48	34	70.87	6	12.50	40	83.3	3	6.25	5	10.41	8	16.66
	10	147	121	82.31	22	14.97	143	97.28	1	0.68	3	2.04	4	2.72
C	11	314	305	97.13	1	0.31	306	97.45	8	2.54			8	2.54
	12	512	496	96.87	12	2.34	508	99.21	1	0.19	3	0.58	4	0.78
	13	288	219	76.04	55	19.09	274	95.13	10	3.46	4	1.38	14	4.86
	14	204	177	86.77	22	10.78	199	97.54	2	0.98	3	1.46	5	2.45
D	15	72	70	97.22	1	1.38	71	98.61			1	1.38	1	1.38
	16	331	263	79.45	36	10.87	299	90.33	3	0.90	29	8.76	32	9.67
	17	70	63	90.00	5	7.14	68	97.14			2	2.85	2	2.85
Totals														
A		1409	1315	93.32	74	5.25	1389	98.58	10	0.70	10	0.70	20	1.41
B		1229	1138	92.68	55	4.48	1193	97.07	9	0.73	27	2.20	36	2.93
C		1318	1197	90.82	90	6.82	1287	97.64	21	1.59	10	0.75	31	2.33
D		473	396	83.72	42	8.88	438	92.6	3	0.63	32	6.76	35	7.39
		4429	4046	91.35	261	5.89	4307	97.24	43	0.97	79	1.78	122	2.75

P—*Positive;* N—*Negative.*

An association coefficient of 0.994 is very high. In only 2.75%* (1.78% + 0.97%) of the diagnoses was there disagreement.

It will be seen from Table II that in 13 of the 17 groups of birds the agreement between the whole blood test and the rapid serum test was over 97%. In the remaining four groups (Nos. 9, 16, 13 and 8) the agreement was 83.3, 90.3, 95.1 and 95.6% respectively.

No particular significance can be attached to the fact that technicians *A*, *B* and *C* obtained an agreement of over 97%, while technician *D* obtained an agreement of 92.6% only. As already stated above, technician *D* supervised the testing of *A*, *B* and *C*, and the final diagnoses were subject to his confirmation.

Although a total disagreement of 2.75% between the diagnosis of the whole blood and the rapid serum tests is not statistically significant, it seemed important to determine the reasons for these differences. Are they due to errors in technique, errors in diagnosis, low agglutinin titre of birds or sensitivity of the antigens *per se*? In order to obtain some light on these questions, retests of birds on which there was disagreement by the whole blood and the rapid serum agglutination tests were undertaken. For this purpose, the birds were collected from the various farms and centralized in one house. Since 20 of the 122 birds upon which there were disagreements in the diagnoses had either been sold or had had their bands removed, only 102 out of the 122 birds were available for retest purposes.

The birds were renumbered and blood samples taken in duplicate, and sent for *retest* to the previously mentioned commercial laboratory (designated hereafter as Laboratory 1), as well as to a second laboratory (Experiment Station Laboratory, hereafter designated as Laboratory 2). Simultaneously retests by the whole blood agglutination test were made by Technician *D*.

The diagnoses obtained with the 102 blood samples on the first test (whole blood test, and rapid serum test, Laboratory 1), and the second test (whole blood test, rapid serum test, Laboratory 1; rapid serum, Laboratory 2) are set forth in Table III.† In order to facilitate a rapid analysis of the data, the results of the various tests are summarized in tabular form.

The diagnoses of the first and second tests by the whole blood test and the rapid serum test, Laboratory 1, are compared in Table IV.

It will be seen from Table IV that a change of diagnosis (positive to negative or *vice versa*) was recorded in 13 cases by the whole blood test and in 32 cases by the rapid serum test. Since the interval between the first and second tests varied from two to nine days, the changes in diagnoses would not probably be due to marked variations in the agglutinin content of the blood sera of the respective fowls. The changes would more likely be due to faulty diagnosis made in either of the two tests.

**This 2.75% is obtained by dividing the 122 cases of disagreement by the total of 4,429 birds of which 4,046 were totally non-reactive, rather than by 383 the total number of reactors to either test. On this latter basis the percentage disagreement is 31.9%.* (Editor's note.)

†*Details may be obtained from the author or the Editor of the Canadian Journal of Research.*

The results of the second tests of the 101 birds which were recorded as disagreements with the first test show that the whole blood test and the rapid serum test, Laboratory 1, agreed in 11 positive diagnoses and in 27 negative diagnoses, a total agreement of 38, or 37.6%. There were three birds in which the diagnoses by both tests were inconsistent. In the case of 60 birds there was still a disagreement in diagnosis between the whole blood test and the rapid serum test, Laboratory 1. This is shown in the following summary:—

—	Whole blood agglutination test	Rapid serum agglutination test, Lab. 1
1. Agreed positives on the 2nd test that were diagnosed as negatives on the 1st test	3	8
2. Agreed negatives on the 2nd test that were diagnosed as positives on the 1st test	6	21

3. Inconsistent diagnoses recorded by both tests:—

Bird No.	Whole blood agglutination test		Rapid serum agglutination test—Lab. 1	
	1st test	2nd test	1st test	2nd test
3077	P	N	N	P
3143	SP	N	N	PP
3167	P	N	N	PP

P, PP = Positive; SP = Suspicious positive; N = Negative.

4. Disagreements between the whole blood test and the rapid serum test, Laboratory 1.

Positive to the whole blood test—negative to the rapid serum test,	15
Negative to the whole blood test—positive to the rapid serum test,	45
Total	60

The disagreement between the whole blood and rapid serum tests recorded in Table II is thus reduced from 122 to 81 (including the 21 birds that were not retested). On the basis of 4,429 birds originally tested, the disagreement is 1.82%.

Table V shows a comparison of the diagnoses by the whole blood test and the rapid serum test (Laboratory 2). It will be seen that both on the first and second tests, twelve birds were recorded as negative to the whole blood test that were positive to the rapid serum test, Laboratory 2. These birds are listed in Table VI.

Of these, three were positive to the first and negative to the second whole blood test, while three were negative to the first but positive to the

TABLE IV

COMPARISON OF DIAGNOSES BY FIRST AND SECOND TESTS

Whole blood agglutination test		Rapid serum agglutination test	
Diagnoses unchanged		*Diagnoses unchanged*	
Positive,	22	Positive,	48
Negative,	67	Negative,	21
Total	89 (87.25%)	Total	69 (68.31%)
Diagnoses changed		*Diagnoses changed*	
Positive to negative,	10	Positive to negative,	21
Negative to positive,	3	Negative to positive,	11
Total	13 (12.74%)	Total	32 (31.68%)

NOTE.—*Inadvertently, one blood sample was not sent to Laboratory 1 for retest, hence total number of blood samples is 101.*

TABLE V

COMPARISON OF DIAGNOSES BY WHOLE BLOOD AND RAPID SERUM AGGLUTINATION TESTS, LABORATORY 2

	W.B.	R.S. Lab. 2	No. of birds on which the diagnosis agreed	—	No. of birds on which the diagnosis disagreed
First test					
Positive	33	21	9	Pos. W. B. to neg. R. S.	23
Negative	69	81	58	Neg. W. B. to pos. R. S.	12
Total	102	102	67		35
Per cent			65.68		43.31
Second test					
Positive	26	21	9	Pos. W. B. to neg. R. S.	17
Negative	76	81	64	Neg. W. B. to pos. R. S.	12
Total	102	102	73		29
Per cent			71.56		28.43

NOTE.—W. B. = *whole blood agglutination test.* R. S. = *rapid serum agglutination test.*

TABLE VI

BIRDS WHICH REACTED NEGATIVE TO FIRST AND SECOND WHOLE BLOOD TESTS, BUT WHICH WERE POSITIVE TO RAPID SERUM AGGLUTINATION TEST, LABORATORY 2

1st test	2nd test
Bird number	
3023	
3029	
3207	
3593	3593
3678	3678
3727	3727
3739	3739
3850	3850
3984	3984
4007	4007
4181	4181
4201	4201
	3077
	3143
	3167

second whole blood test. Of the 21 birds that reacted positive to the rapid serum test (Laboratory 2), 9 failed to react to both the first and second whole blood tests. The failure of these nine birds to react positively to the whole blood test cannot be attributed to error in technique, but rather to lack of sensitivity of the antigen or low agglutinin content of the sera. On the other hand, 23 birds (1st test) and 17 (2nd test) were diagnosed positive by the whole blood, but negative by the rapid serum test (Laboratory 2).

The data in Table VII show that the first rapid serum test (Laboratory 1) diagnosed as negative nine birds that were positive to the rapid serum test (Laboratory 2). On the second test, the rapid serum test (Laboratory 1) showed as positive all the birds that were diagnosed as positive by the rapid serum test (Laboratory 2). But, since Laboratory 1 diagnosed a number of birds as positive that were negative to the Laboratory 2 test, the disagreement between the first and second rapid

serum tests (Laboratory 1) and the rapid serum test (Laboratory 2), was 65.34 and 37.62% respectively.

In comparing Tables V and VII it will be seen that the agreement between the diagnoses of the first and second whole blood tests and the rapid serum test (Laboratory 2), was 65.65 and 71.5% respectively, while the agreement between the first and second rapid serum tests (Laboratory 1) and the rapid serum test (Laboratory 2) was 34.65 and 62.3% respectively. There was thus a closer agreement between the whole blood test and the rapid serum test (Laboratory 2) than between the rapid serum test (Laboratory 1) and the rapid serum test (Laboratory 2).

TABLE VII

COMPARISON OF DIAGNOSES BY RAPID SERUM AGGLUTINATION TESTS BY LABORATORY 1 AND LABORATORY 2

	Lab. 1	Lab. 2	No. of birds on which the diagnosis agreed	—	No. of birds on which the diagnosis disagreed
First test					
Positive	69	21	12	Pos. Lab. 1 to Neg. Lab. 2	57
Negative	32	80	23	Neg. Lab. 1 to Pos. Lab. 2	9
Total	101	101	35		66
Per cent			34.65		65.34
Second test					
Positive	59	21	21	Pos. Lab. 1 to Neg. Lab. 2	38
Negative	42	80	42	Neg. Lab. 2 to Pos. Lab. 1	0
Total	101	101	63		38
Per cent			65.34		37.62

Post-mortem Examination

To obtain further information regarding the relative diagnostic value of the whole blood agglutination test and the rapid serum agglutination test as conducted by Laboratory 1 and Laboratory 2, the birds were killed and a careful post-mortem examination was made. Eighty-eight of the birds were examined by the senior author, and 12 birds, by Laboratory 2, while two birds that died from intervening causes were not examined. The results of the post-mortem examination are shown in Table III,* together with the agglutination test diagnoses of the birds that were retested. Since Laboratory 2 conducted additional agglutination tests with the 12 birds, the results of the retests and post-mortem examination are shown separately in Table VIII. The post-mortem examinations were conducted according to the technique described in a previous paper (2).

* See second footnote p. 800.

From a consideration of Table III* it was observed that out of 21 birds that were diagnosed as positive by the tube and rapid serum agglutination tests (Laboratory 2), *S. pullorum* was isolated from 13 birds, or 57.1%. *S. pullorum* was not isolated from any of the birds found positive by Laboratory 1, but negative by Laboratory 2. With the exception of two cases *S. pullorum* was not isolated from any of the birds diagnosed positive by the whole blood agglutination test but negative by Laboratory 2.

The comparatively small percentage of birds from which *S. pullorum* was isolated was due to the fact that out of the 102 birds in which there was a disagreement between the whole blood agglutination test and the rapid serum agglutination test (Laboratory 1), 81 birds were apparently negative (as shown by Laboratory 2), while of the remaining 21 positive birds, several were borderline or doubtful reactors. This is clearly brought out in Table VIII, which shows that not one of the six birds which were diagnosed as positive by Laboratory 2 rapid serum and tube agglutination tests gave a definite reaction in a 1 : 100 dilution by the tube agglutination test. Furthermore, these six birds showed slight fluctuations in reaction from test to test.

TABLE VIII

DETAILS OF RETESTS ON TWELVE BIRDS AUTOPSIED BY LABORATORY 2

Number of bird	Whole blood		Rapid serum—Lab. 1			Rapid serum—Lab. 2	Rapid serum—Lab. 2	Tube 1 : 100 dil.—Lab. 2	Rapid serum—Lab. 2	Rapid serum—Lab. 2	Tube 1 : 100 dil.—Lab. 2	Rapid serum—Lab. 2	Rapid serum—Lab. 2	Tube 1 : 100 dil.—Lab. 2	Diagnosis—Lab. 2	Post-mortem findings: Macroscopic	Post-mortem findings: Bacteriological
	1	2	1	2	3	Sept. 30, 1933			Oct. 17, 1933			Oct. 25, 1933					
2910	P	P	N	N	—	—			—	—	—	—	—	—	N	No lesions	Negative
2954	P	P	N	N	—	—			—	—	—	—	—	—	N	No lesions	Negative
3023	N	P	P	P	†††	†††	†††	†††	†††	†††	†††?	†††	†††	††?	P	No lesions	Negative
3207	N	N	P	P	††	†††	†††	††	††	††	—?	††?	††	—?	P	Two typical ova; one suspicious	*S. pullorum*
3305	P	P	N	N	†	—			††?	††?	—	—	—	—	N	No lesions	Negative
3487	P	N	N	N	—	—			—	—	—	—	—	—	N	No lesions	Negative
3510	P	N	N	N	—	—			—	—	—	—	—	—	N	No lesions	Negative
3573	P	N	N	N	—	††?	†?	—?	—	—	—	—	—	—	N	No lesions	Negative
3727	N	P	N	P	††	†††	††	†††?	†††?	†††?	†	††	††	††?	P	Two typical ova; two suspicious	*S. pullorum*
3739	N	P	P	P	†††	†††	†††	†††	†††	†††	†††	†††	†††	†††?	P	No lesions	Negative
3850	N	N	P	P	†††	†††	†††	†††	†††	†††	††?	†††	†††	††?	P	No lesions	Negative
3984	N	N	P	P	††	†††	†††	†††	†††?	††	††	††	††	††	P	Five typical ova	*S. pullorum*

NOTE.—*1—Routine test. 2—Retest. 3—Second retest. P = Positive. N = Negative. †††, †† = Positive. †, — = Negative. ? = Doubtful.*

**See second footnote on p. 800.*

Discussion

The present study shows that on the basis of the first routine whole blood agglutination test, 12 birds, and on the basis of the first rapid serum agglutination test, 9 birds, that were subsequently shown to be positive by the rapid serum agglutination test (Laboratory 2) were diagnosed as negative birds. The remaining disagreements were due to birds that were diagnosed by the whole blood and rapid serum agglutination tests (Laboratory 1) as positive, but as negative by the rapid serum agglutination test (Laboratory 2). The fact that the whole blood and rapid serum agglutination tests failed to detect a total of 21 positive birds is more important than the fact that 81 birds that were diagnosed as positive by the whole blood and rapid serum (Laboratory 1), were negative according to the test of Laboratory 2. With regard to control or eradication of pullorum disease, the disagreement in the diagnoses of positive birds between the whole blood and rapid serum agglutination tests was 21 out of 4,429 birds, or 0.47%.*

Considerable work has already been done to show that the serum test is an accurate and valuable means of diagnosing pullorum disease carriers. While it is true that the serum test permits a more exact diagnosis of pullorum disease, both the rapid and the tube tests are nevertheless subject to error. In the final analysis, the accuracy of any laboratory test depends upon the person performing it. The main objection to the whole blood agglutination test is the general claim that it is not sufficiently standardized. The studies of the writers and those of other investigators, show that the antigen is very stable and uniform in quality, while the technique is comparatively simple. The only variable is the personal factor in the interpretation of the results of the reaction. It should not be any more difficult to train men to perform the whole blood agglutination test than to perform the serum agglutination tests.

Since this and previous studies show that there is a close agreement in the diagnoses made on the basis of the whole blood and either the tube or rapid serum agglutination tests, the usefulness of the whole blood agglutination test can no longer be questioned. Furthermore, since it is more adaptable to mass application, its value to poultrymen and to the industry as a whole is so much greater. Since it lends itself so admirably to repeated application at short intervals, it is more practical than either of the other tests.

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**This percentage would be much higher in a heavily infected flock. Assuming, for example, that in the case under consideration all the birds reacting to both tests were affected by the disease, the total number of affected birds was 261 plus 21. The whole blood test left in the flocks 12 birds which constituted a hazard to the remainder, which the rapid serum test left 9 birds. Viewed from the point of diagnosis of the positive birds, therefore, the tests disagreed in a total of 21 out of 282, or 7.4% (instead of 0.47% when the negative birds were also included).* (Editor's note.)

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